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(54) Title: COMPOUND SCREENS RELATING TO INSULIN DEFICIENCY OR INSULIN RESISTANCE

(57) Abstract: The invention is concerned with use of the model organism *C. elegans* as a research tool to screen for compounds active in insulin signalling. In particular, the invention relates to improved screening methods based on release of *C. elegans* from the dauer larval state.

COMPOUND SCREENS RELATING TO INSULIN DEFICIENCY OR INSULIN RESISTANCE

The present invention is concerned with using the model organism *C. elegans* as a research tool to effectively screen compound libraries for compounds active in insulin signalling, in particular compounds which act downstream of the insulin receptor. Specifically the invention relates to improved screening methods based on release of *C. elegans* from the dauer larval state.

In a particular embodiment, the invention provides improved screening methods using *C. elegans* carrying mutations in one or more gene(s) involved in the insulin signalling pathway, such as the Daf-genes. In one particular embodiment, (at least one of) said mutation(s) is in the *daf-2* gene, which is homologous to the insulin receptor subfamily of receptor tyrosine kinases. One the basis of the homology between *daf-2* and the insulin receptor subfamily it is proposed that worms mutant in the *daf-2* gene may serve as models for insulin-related diseases and disease risks, as for example diabetes mellitus, obesity, insulin resistance and impaired glucose tolerance (Kimura et al. 1997, Science 277, 942-946).

General techniques and methodology for performing in vivo assays using the nematode worm *Caenorhabditis elegans* (*C.elegans*) as a model organism have been described in the art, most notably in the following applications by applicant: PCT/EP99/09710 (published on 15 June 2000 as WO 00/34438); PCT/EP99/04718 (published on January 15, 2000 as WO/00/01846);

PCT/IB00/00575 (published on October 26, 2000 as WO 00/63427); PCT/IB00/00557 (published on October 26, 2000 as WO 00/63425); PCT/IB00/00558 (published on October 26, 2000 as WO 00/63426); as well as for instance PCT/US98/10080 (published on 19-11-1998 as WO 98/51351), PCT/US99/13650, PCT/US99/01361 (published on 29-07-1999 as WO99/37770), and PCT/EP00/05102.

As described in these applications, one of the main advantages of assays involving the use of *C. elegans* is that such assays can be carried out in multi-well plate format (with each well usually containing a sample of between 2 and 100 worms) and - also because of this - may also be carried out in an automated fashion, i.e. using suitable robotics (as are described in the aforementioned applications and/or as may be commercially available). This makes assays involving the use of *C. elegans* ideally suited for screening of libraries of chemical compounds, in particular at medium to high throughput. Such automated screens may for instance be used in the discovery and/or development of new compounds (e.g. small molecules) for pharmaceutical, veterinary or agrochemical/ pesticidal (e.g. insecticidal and/or nematocidal) use.

Some other advantages associated with the use of *C.elegans* as a model organism (e.g. in the assay techniques referred to above) include, but are not limited to:

- *C. elegans* has a short life-cycle of about 3 days. This not only means that these nematodes (and suitable mutants, transgenics and/or stable lines thereof) can

be cultivated/generated quickly and in high numbers, but also allows assays using *C.elegans* to test, in a relatively short period of time and at high throughput, the nematode worms over one or more, and up to all, stages of life/development, and even over one or more generations. Also, because of this short life span, in *C.elegans* based-assays, compounds may be tested over one or more, and up to essentially all, stages of development, without any problems associated with compound stability and/or (bio)availability;

- *C. elegans* is transparant, allowing -with advantage- for visual or non-visual inspection of internal organs and internal processes, and also the use of markers such as fluorescent reporter proteins, even while the worms are still alive. Also, as further mentioned below, such inspection may be carried out in automated fashion using suitable equipment such as plate readers;

- *C.elegans* is a well-established and well-characterized model organism. For example, the genome of *C.elegans* has been fully sequenced, and also the complete lineage and cell interactions (for example of synapses) are known. In addition, *C.elegans* has full diploid genetics, and is capable of both sexual reproduction (e.g. for crossing) as well as reproduction as a self-fertilizing hermaphrodite. All this may provide many advantages, not only for the use of *C.elegans* in genetic and/or biological studies, but also for the use of *C.elegans* in the discovery, development and/or pharmacology of (candidate) drugs

for human or animal use.

- Techniques for transforming, handling, cultivating, maintaining and storing (e.g. as frozen samples, which offers great practical advantages) *C. elegans* are well established in the art, for instance from the handbooks referred to below. For example, *C.elegans* may be used as one or more samples with essentially fully isogenic genotype(s).

Generally, in the assays described above, the nematodes are incubated in suitable vessel or container - such as a compartment or well of a multi-well plate - on a suitable medium (which may be a solid, semi-solid, viscous or liquid medium, with liquid and viscous media usually being preferred for assays in multi-well plate format). The nematodes are then contacted with the compound(s) to be tested, e.g. by adding the compound to the medium containing the worms. After a suitable incubation time (i.e. sufficient for the compound to have its effect - if any - on the nematodes), the worms are then subjected to a suitable detection technique, i.e. to measure/determine a signal that is representative for the influence of the compound(s) to be tested on the nematode worms, which may then be used as a measure for the activity of the compound(s) in the in vivo assay.

Often, in particular for automated assays, such a detection technique involves a non-visual detection method (as further described in the applications mentioned above), such as measurement of fluorescence

or another optical method, measurement of a particular marker (either associated with worms or associated with the medium) such as autonomous fluorescent proteins (AFP's) such as green fluorescent proteins (GFP's), aequorin, alkaline phosphatase, luciferase, Beta-glucoronidase, Beta- lactamase, Beta-galactosidase, acetohydroxyacid, chloramphenicol acetyl transferase, horse radish peroxidase, nopaline synthase, or octapine synthase. For example, for automated assays carried out in multi-well plates, so called (multi-well) "plate readers" may be used for detecting/measuring said signal.

For a further description of the above and other assay techniques involving the use of nematodes as a model organism, reference is made to the prior art, such as the applications by applicant referred to above.

For general information on *C.elegans* and techniques for handling this nematode worm, reference is made to the standard handbooks, such as W.B. Wood et al., "*The nematode Caenorhabditis elegans*", Cold Spring Harbor Laboratory Press (1988) and D.L. Riddle et al., "*C. ELEGANS II*", Cold Spring Harbor Laboratory Press (1997).

The use of *C.elegans* based assays in the field of metabolic diseases - such as obesity and diabetes - has been described in a number of applications, most notably in PCT US 98/10800 and US-A-6,225,120, which relate to the use of daf-2 mutant *C.elegans* nematodes for selecting compounds active in impaired glucose tolerance and diabetes, as a model for insulin resistance.

One of the main objects of the present invention is to provide improved methods for the selection of compounds for the field of metabolic diseases - including but not limited to obesity, impaired glucose tolerance and type-II diabetes - which methods may be used for drug discovery, development, pharmacology and testing. In particular, it is an object of the invention to provide such improved assays as compared to the assay techniques described in PCT US 98/10800 and US-A-6,225,120.

Generally, the invention solves this problem by the use, in such assays, of nematode strains (such as m41) which have increased sensitivity of the insulin signalling pathway compared to the strains used in PCT US 98/10800 and US-A-6,225,120.

Diabetes mellitus is a major growing public health problem in both developed and developing countries. Including clinical complications it accounts for 5% of the total healthcare expenditure in Europe. Depending on the type of diabetes, current drug therapy strategy for diabetes consist of a diet supported by either application of exogenous insulin of different origin, application of drugs that increase production and/or release of endogenous insulin, enhance sensitivity of peripheral organs to insulin or mimic insulin effects. Drugs acting directly in the insulin pathway downstream of the receptor are potentially beneficial in both major types of diabetes but they are not existing today. The major drawback of currently available drugs is the body weight gain that comes on top of an existing obesity in the vast majority (80%) of patients. This

side effect is also the main reason why pharmacological intervention in the middle range of disease development is not as intense and aggressive, as it should be to achieve optimal efficacy. New drugs that are devoid of this side effect would already reduce risk of complications by 12 to 30% (United Kingdom prospective diabetes study. Turner et al. 1998, BMJ 316: 823-828; Turner et al. 1999, JAMA 281: 2005-2012).

Novel glitazones, such as troglitazone, that act on nuclear receptors which regulate carbohydrate metabolism that have been launched in Japan and the US were withdrawn due to an elevated risk of liver toxicity. Hence the medical need for well tolerated orally-active anti-diabetics with mild benign side-effects remains high. A compound that directly interacts downstream the insulin receptor pathway could establish a breakthrough especially since it could be a drug that acts both in Type I and Type II diabetes. A compound that has as a clinical result an insulin sparing effect could also be of extremely high therapeutic value.

From animal studies inorganic vanadates are known to favourably combine increase in insulin sensitivity and reduction of hyperlipidemia together with body weight stability or loss, but are devoid of body weight gain (Brichard and Henquin 1995, TiPS 16: 265-270). Due to unresolved toxicity issues, however, they are not available in drug formulas. Although inorganic vanadium compounds are currently in clinical trial, the issue of side effects still raises doubts for this class of compounds to have to specification

of a drug, which has to be well tolerated in multiple doses per day for decades.

Nevertheless, the recognition of protein tyrosine phosphatase 1B as the major target of vanadates and the validation of this target as strongly increasing insulin sensitivity when inactivated in mice points towards the insulin receptor pathway as valuable for finding active compounds to ameliorate insulin resistance (Elchebly et al. 1999, Science 283: 1544-1548). PTP-1B is a negative regulator of insulin receptor tyrosine phosphorylation and kinase activity, its inactivation is raising insulin signalling with given constant insulin levels (Figure 1). The present inventors have shown that vanadates can rescue the genetic insulin resistance caused by daf-2 mutations in *Caenorhabditis elegans*, thereby validating the genetic model for insulin-deficient and insulin-resistant related disease by pharmacological means (Figure 3). Wortmannin, an inhibitor of the downstream effector phosphatidyl-inositol-3-phosphat kinase (Figure 1), further increases insulin resistance, confirming the sensitivity of the invented assay for the pathway (Figure 4). The possible known targets in the insulin-receptor pathway shown in Figure 1 are listed in table 1.

The inventors have made two key adaptations which enable them to use *C. elegans* mutant strains to effectively screen large compound libraries for activities mimicking vanadates using screens based on rescue of the phenotype dauer formation and other phenotypic traits which are caused by interventions in the insulin signalling pathway, such as, for example,

mutations in the insulin receptor gene homologue *daf-2*. The first adaptation is the use of *C. elegans* with a sensitized genetic background; the second adaptation is manipulation of the assay conditions such that a basal level of release from the dauer larval state is present even in the absence of test compounds. The *daf-2* gene had previously been disregarded as useful target for compound screens due to a failure of obtaining active compounds from large compound libraries (Carl Johnson, Axys pharmaceuticals, Nemapharm division, disclosed at the Cold Spring Harbor worm course). The new developments described herein overcome sensitivity problems previously encountered with screens based on *daf-2*.

In the invention, generally nematode strains are used that show sensitivity of the insulin signalling pathway.

In particular, these strains are used in assays involving the use of a dauer stage and/or dauer phenotype as a read out. These may for instance be assays based on "dauer rescue" and/or on "dauer formation/bypass" (of which dauer bypass is usually preferred, as it may avoid the problems associated with the limited uptake of the compound(s) to be tested by worms in the dauer state).

In the former type of assay, a sample of worms in the dauer state is provided, and the efficacy of the compound(s) to be tested in bringing the worms of said sample out of the dauer state is determined. Generally, compounds with the desired activity will bring the worms out of the dauer state (i.e. to a greater degree than a reference without compound, and

preferably in a dose/concentration-dependant manner) and thus provide adults (i.e. more adults than without the presence of the compound(s) to be tested).

In the latter type of assay, a sample of worms (in particular eggs, L1 or 12 worms, and preferably L1 worms) is kept under conditions which, without the presence of any compound(s) to be tested, would cause (most and preferably essentially all) of the worms in the sample to enter the dauer state, and the efficacy of the compound(s) to be tested in preventing the worms, under these conditions, to enter the dauer state (i.e. to bypass the dauer state) is determined. Generally, compounds with the desired activity will prevent the worms from entering the dauer state (i.e. to a greater degree than a reference without compound, and preferably in a dose/concentration-dependant manner) and thus provide adults (i.e. more adults than without the presence of the compound(s) to be tested, and preferably in a dose-dependant manner). Conditions such that the worm strain(s) used will enter the dauer state without the presence of the compound(s) to be tested will depend on the specific worms strain used and will be clear to the skilled person, also in view of the preferred conditions described hereinbelow. Also, these conditions are preferably such that, under the conditions of the assay, a reference compound with the desired activity (such as vanadate at a concentration of between 0.5 and 2 milliMolar) will allow a measurable amount of worms to bypass the dauer state (e.g. between 40 to 70%, or even more). If necessary, the results obtained with such a reference compound may also serve as a positive control or

comparative reference for the compound(s) to be tested.

As will be clear to the skilled person, for both the dauer rescue and the dauer bypass assays described above, and during or at the end of the assay, either the number of dauer larvae in the sample and/or the number of adults may be determined (with the sum of the number of dauer larvae and the number of adults being essentially equal to the number of worms present in the original sample). Techniques for determining the number of adults and/or dauer larvae in a sample will be clear to the skilled person and may include visual inspection of the sample (e.g. counting) as well as the automated non-visual detection techniques referred to above.

In the context of the present invention, the insulin signalling pathway may generally be described in all enzymatic conversions and other signal transduction events that are involved in (transmembrane) receptor-mediated (cellular) signal transduction in response to the (extracellular) presence insulin signals (e.g. the extracellular presence of insulin or insulin-like compounds). Some of the most important (but non-limiting) examples of the different enzymatic conversions involved in said signalling have already been mentioned hereinabove.

By "*sensitivity of the insulin signalling pathway*" is generally meant that

1) the nematode shows one or more biological response(s) to the presence of an insulin, to the presence of an insulin-like compound, and/or to the presence of a compound that can provide and/or or

mimic a biological response similar to the biological response(s) provided by insulin or the insulin-like molecules (which three categories are also collectively referred to herein as "*insulin-like signals*"); and that

2) said one or more biological responses change when (the amount of) the compound(s) to which the nematode is exposed (and/or with which said nematode comes into contact) changes or is altered (for instance, due to a change in the concentration of said insulin like signal in the medium.

The biological response may be any response or combination of responses, such as one or more changes in physiology, biochemistry, development, behaviour, exitation, or other phenotypical properties.

In one particularly preferred embodiment, these may essentially be one or more of the biological responses that are (also) obtained upon (over)expression of insulin the nematode.

One particularly suited biological response may be the dauer-behaviour, e.g. the entry, exit, rescue or bypass of the dauer state, and/or other phenotypical properties that result from and/or are associated with the so-called dauer decision.

In the invention, (one or more strains of) nematodes are used that show increased sensitivity of the insulin pathway, compared to at least the wildtype, and preferably also compared to the reference strain CB1370 (containing the *daf-2* reference mutation e1370. This strain is publicly available, for example from the Caenorhabditis Genetics Center (CGC), Minnesota, USA).

By "*increased sensitivity of the insulin signalling pathway*" is generally meant that the change in the biological response of the nematode (as described above) to a change in (the concentration of) the insulin-type signal is greater than the change that is obtained with the wildtype and/or CB1370 (i.e. for the same change in (the concentration of) the insulin-type signal).

For example, when a change in (e.g. an increase or reduction of) the concentration of an insulin-type signal gives, for the wildtype and/or CB1370, a change in (e.g. an increase or reduction of) the biological response of by a factor of *x*, than the same change will give, for a strain suitable for use in the invention, a change in the same biological response of more than *x* (e.g. 1.05 times x, preferably 1.1 times x, more preferably 1.5 times x or even 2 times x or 10 times x, depending on the biological response, the insulin-type signal, the change in concentration, and the specific strain(s) used). In case there is no change observed in wildtype and/or the reference strain CB1370, any change observed determines a strain to be of "*increased sensitivity to a insulin-type signal*".

For example, an "*insulin-type signal*" as used herein may be:

- an insulin or insulin-like molecule (e.g. from any suitable source, including but not limited to nematodes, humans or other animals), for which reference is made to PCT/US99/08522, published as WO99/54436 on 28.10.99; Genes & Development 15:672-686,2001;

- a vanadate or a vanadate-type compound, such as sodium orthovanadate;
- a PTB-1B inhibitor such as described in Journal of Medicinal Chemistry 43:1293-1310,25.02.2000, for example compound 66;
- wortmannin or a wortmannin-type compound, such as LY 294002 or other PI3-kinase inhibitors.

In this respect, it should be noted that an increase in the concentration of an insulin-type signal may provide an increase in the biological response (in which said increase will be more pronounced for the strain of the invention than for the wildtype and/or for CB1370), or may provide a decrease in the biological response (in which said decrease will be more pronounced for the strain of the invention than for the wildtype and/or for CB1370). For example, an increase in the concentration of a wortmannin will provide an increase in the biological response (for example more dauer), which will be even more pronounced for the strains of the invention (e.g. even more dauer compared to wildtype/CB1370 per increased concentration of wortmannin), whereas an increase in the concentration of a vanadate will provide a decrease in the biological response (for example less dauer), which will be even more pronounced for the strains of the invention (e.g. even less dauer compared to wildtype/CB1370 per increased concentration of vanadate). In case the number of nematodes grown up, i.e. non-dauer, are counted, positive (i.e. increased) and negative (i.e. decreased) biological response are reversed into each other. Both types of insulin-type signals may be used

for to determine whether a specific nematode strain has "*increased sensitivity of the insulin signalling pathway*" compared to wildtype and/or CB1370, and which may be used within the scope of the present invention.

Preferably, the insulin-type signal that is used to determine whether a specific nematode strain has "*increased sensitivity of the insulin signalling pathway*" is a vanadate-type compound. The vanadate may be used as a free base or as a suitable water-soluble salt, such as sodium orthovanadate. Preferably, the vanadate is used in an amount of between 0.01 and 100 millimolar, more preferably between 0.1 and 10 millimolar, such as 0.5 millimolar or 2.0 millimolar.

Some specific conditions under which vanadates may be used to determine whether a specific nematode strain has "*increased sensitivity of the insulin signalling pathway*" will be further described below.

Thus, as will be clear from the above, the "insulin-type factor(s)" described above may be used to determine whether a strain has increased sensitivity of the insulin signalling pathway (i.e. compared to the wildtype and/or CB1370) and thus may be used within the scope of the invention.

Generally, such a nematode strain useful in the invention will have "*increased sensitivity of the insulin signalling pathway*" due to a mutation and/or an other genetically determined factor that provides such increased sensitivity. Such strains will also be referred to below as having a "sensitized genetic background", and some preferred examples thereof, such as DR1564 and CB1368, will be further described below.

However, it is also within the scope of the invention to provide the strain(s) used with "increased sensitivity of the insulin signalling pathway" by other means, such as exposure to pheromones which increase such sensitivity, by gene suppression techniques such as RNAi, and/or by growing/cultivating the nematodes in the presence of an inducing or suppressing factor (such as population density, food concentration and temperature).

In particular, the nematode strain used may be a weak Daf mutant (i.e. a mutation abnormal in dauer formation), in particular a Daf mutant that is weaker then the reference strain CB1370. For instance, it may be a *age-1* mutant, or one of the other *daf* mutants mentioned herein.

In particular, the nematode strain used may be a weak *daf-2* mutant, in particular a *daf-2* mutant that is weaker then the reference strain CB1370.

For instance, the reference strain used may be have a Class-I mutation (as mentioned in Gems et al., supra), a mutation which provides a phenotype similar to - and preferably essentially the same as - a Class-I mutation, and/or a(nother) mutation in the ligand binding domain, such that the mutated receptor still has an active kinase domain, but the sensitivity to insulin-like signalling is impaired. However, in its broadest scope, the invention is not limited thereto, and other mutations may also be present, including Class II mutations, as long as the strain having the mutation still has increased sensitivity of the insulin signalling pathway, compared to the wildtype and/or the reference strain *C. elegans* CB1370.

It is also possible, in the assays of the invention, to use two or more different strains, e.g. one or more which have increased sensitivity of the insulin signalling pathway, and/or one or more references, e.g. wildtype or CB1370.

In one preferred, but non-limiting aspect of the invention, the sensitivity of the insulin signalling pathway of the nematode strain used may be expressed in terms of the "*Insulin Sensitivity Value*" (ISV), which may be determined in the following manner:

A sample of nematode worms (preferably in the L1 stage) is incubated for between 48 and 96 hours (preferably about 72 hours) separately with and without an insulin-type signal (preferably a vanadate-type compound), at a temperature of between 20 and 25°C (such as 20, 21, 22, 23, 24 or 25°C), in the presence of a suitable source of food (such as bacteria, e.g. between 0.05 and 0.5 % w/v, preferably about 0,125 % w/v), and using a suitable medium (such as S-buffer, M9 or one of the media described in the applications referred to above, and preferably S-buffer).

After incubation, for both the sample with the insulin-type signal and the sample without the insulin-type signal compound, the number of worms in the sample that enter into the dauer state is determined, as a percentage of the number of worms in the original sample, i.e. as follows:

1) for the sample without the insulin-type signal:
([*the number of worms that enter the dauer state without insulin-type signal*] divided by [*the*

total number of L1 worms in the original sample]) times [*100%*].

This percentage is herein referred to as "Percentage A".

2) for the sample with the insulin-type signal:

([*the number of worms that enter the dauer state with the insulin-type signal*] divided by [*the total number of L1 worms in the original sample*]) times [*100%*].

This percentage is herein referred to as "Percentage B".

The Insulin Sensitivity Value may then be expressed as the absolute difference between "Percentage A" and "Percentage B" (i.e. as absolute value of ["Percentage A" minus "Percentage B"]).

As the ISV is calculated as a difference between two percentages A and B, the ISV itself will be a percentage (for instance, when Percentage A is 90%, and percentage B is 10%, the ISV will be 90% - 10% = 80%), and always positive as the absolute value is calculated (for instance, when Percentage A is 10% and Percentage B is 90%, the ISV will be |10% - 90%| = |-80%| = 80%.

In the invention, the nematode strain used preferably has an ISV that is greater than the ISV for CB1370. In particular, the nematode strain used may be such that its ISV is more than 1% greater, preferably more than 5% greater, more preferably more than 10% greater, even more preferably more than 20% greater than the ISV for CB1370 (e.g. calculated as the absolute difference between the ISV for the strain

used and the ISV for CB1370, e.g. [*ISV strain used*] minus [*ISV CB1370*]).

For example, depending upon the specific conditions of the test, CB1370 will usually have an ISV of <20%, more usually <10%, and often <5% (in essence, this means that under the conditions of the test, for CB1370, there is little no difference between the presence and the absence of the insulin type signal). The ISV for wildtype will usually be even lower than the ISV for CB1370.

For the strain used in the invention, under the same conditions of the test, the ISV will usually be >30 %, and is preferably >40%, and is even more preferably >50%. (in essence, this means that under the conditions of the test, for the strain used, the difference between the presence and the absence of the insulin-type signal is preferably (much) larger than for CB1370).

Preferably, the ISV is determined using a vanadate-type compound such as sodium orthovanadate, although the invention in its broadest sense is not limited thereto.

Thus, by determining the ISV in the manner outlined above, it can be determined whether a strain has increased sensitivity of the insulin signalling pathway, compared to the wild-type and/or the reference strain CB1370.

Generally, the invention is based on the insight that such nematode strains having increased sensitivity of the insulin signalling pathway can be used with advantage to provide improved methods for the selection of compounds for the field of metabolic

diseases, in particular compared to the assay techniques described in PCT US 98/10800 and US-A-6,225,120. As mentioned above, these methods may be used for drug discovery, development and pharmacology, for instance to discover and/or develop new small molecules and/or small peptides suitable for use in preventing or treating metabolic diseases in human or vertebrates (such as mammals).

For the purposes of the present disclosure, a "small molecule" generally means a molecular entity with a molecular weight of less than 1500, preferably less than 1000. This may for example be an organic, inorganic or organometallic molecule, which may also be in the form or a suitable salt, such as a water-soluble salt.

The term "small molecule" also covers complexes, chelates and similar molecular entities, as long as their (total) molecular weight is in the range indicated above.

In a preferred embodiment, such a "small molecule" has been designed according, and/or meets the criteria of, at least one, preferably at least any two, more preferably at least any three, and up to all of the so-called Lipinski rules for drug likeness prediction (vide Lipinksi et al., Advanced Drug Delivery Reviews 23 (1997), pages 3-25). As is known in the art, small molecules which meet these criteria are particularly suited (as starting points) for the (design and/or) development of drugs (e.g) for human use, e.g. for use in (the design and/or compiling of) chemical libraries for (high throughput screening), (as starting points for) hits-to-leads chemistry,

and/or (as starting points for) lead development.

In a preferred embodiment, such a "small molecule" has been designed according, and/or meets the criteria of, at least one, preferably at least any two, more preferably at least any three, and up to all of the so-called Lipinski rules for rational drug design (vide Lipinksi *et al.*, Advanced Drug Delivery Reviews 23 (1997), pages 3-25). As is known in the art, small molecules which meet these criteria are particularly suited (as starting points for) the design and/or development of drugs (e.g) for human use

Also, for these purposes, the design of such small molecules (as well as the design of libraries consisting of such small molecules) preferably also takes into account the presence of pharmacophore points, for example according to the methods described by I. Muegge et al., J. Med. Chem. 44, 12 (2001), pages 1-6 and the documents cited herein.

The term "small peptide " generally covers (oligo)peptides that contain a total of between 2 and 35, such as for example between 3 and 25, amino acids (e.g. in one or more connected chains, and preferably a single chain). It will be clear that some of these small peptides will also be included in the term small molecule as used herein, depending on their molecular weight.

Thus, the methods of the invention may in particular be used to test and/or screen (libraries of) such small molecules and/or peptides, in the manner as further outlined herein.

Thus, in one aspect, the invention relates to the use of at least one nematode worm which has an

increased sensitivity of the insulin signalling pathway (compared to the wildtype and/or the reference strain CB1370), in an assay for the identification of a compound, such as a small molecule and/or a small peptide, which is capable of modulating insulin signalling pathways (for example in *C. elegans* and/or vertebrates, such as humans and/or other mammals), more generally of altering and/or effecting the biological response to insulin signalling, and even more generally for use in (the preparation of compositions for) the prevention and/or treatment of metabolic diseases or disorders (as mentioned above), in vertebrates such as humans or other mammals.

In addition to the identification of small molecules and/or small peptides, according to the inventions, the nematode worms with an increased sensitivity of the insulin signalling pathway may also be used for determining the influence or effect of gene suppression (e.g. by RNAi techniques), and of specific or non-specific mutations (e.g. due to non-specific or (site-)specific mutagenesis).

Preferably, the nematode worm with increased sensitivity of the insulin signalling pathway has a sensitized genetic background (compared to the wildtype and/or the reference strain CB1370), as defined above.

Even more preferably, the nematode worm with increased sensitivity of the insulin signalling pathway (e.g. a sensitized genetic background) has an ISV which is greater than the ISV for wildtype and/or CB1370, and even more preferably an ISV as defined above.

Some preferred, but non limited examples of suitable *C. elegans* strains include, but are not limited to: DR1564: *daf-2(m41)*, CB1368: *daf-2(e1368)* and some of the (other) strains mentioned in Gems et al., supra. Other suitable strains will be clear to the skilled person, based upon the disclosure herein.

The most preferred nematode strain is DR1564: *daf-2(m41)*.

The sample of nematodes may comprise any suitable number of worms, depending on the size of the container/vessel used. Usually, the sample will comprise between 2 and 500, in preferably between 3 and 300, more preferably between 5 and 200, even more preferably between 10 and 100 nematodes. When the assay is carried out in multi-well plate format, each well usually contains between 15 and 75 worms, such as 20 to 50 worms. Although not preferred, it is not excluded that a sample may consist of a single worm.

Usually, each such individual sample of worms will consist of worms that - at least at the start of the assay - are essentially the same, in that they are of the same strain, in that they contain the same mutation(s), in that they are essentially of an isogenic genotype, in that they show essentially the same phenotype(s), in that they are essentially "synchronised" (i.e. at essentially the same stage of development, such as L1 or dauer. It should however be noted that this stage of development may - and usually will - change during the course of the assay, and not for all worms in the sample at the same rate and/or in the same way), in that they have been grown/cultivated in essentially the same way, and/or in that they have

been grown under and/or exposed to essentially the same conditions, factors or compounds, including but not limited to pheromones, gene suppression (such as by RNAi), gene- or pathway-inducing factors or (small) molecules, and/or gene- or pathway-inhibiting factors or (small) molecules. However, in its broadest sense, the invention is not limited thereto.

The medium may further contain all factors, compounds and/or nutrients required to carry out the assay and/or required for the survival, maintenance and/or growth of the worms. For this, reference is again made to the prior art, such as the applications and handbooks referred to above. In one specific embodiment, the medium may also contain a suitable source of food for the worms - such as bacteria (for example a suitable strain of *E. coli*) - in a suitable amount.

In the method of the invention, the sample of nematodes can be kept - e.g. maintained, grown or incubated - in any suitable vessel or container, but is preferably kept in a well of a multi-well plate, such as standard 6, 24, 48, 96, 384, 1536, or 3072 well-plates (in which each well of the multi-well plate may contain a separate sample of worms, which may be the same or different). Such plates and general techniques and apparatus for maintaining/ handling nematode worms in such multi-well plate format are well known in the art, for instance from the applications mentioned hereinabove.

The sample of nematodes may be kept in or on any suitable medium - including but not limited to solid and semi-solid media - but is preferably kept in a

suitable liquid or viscous medium (e.g. with a viscosity at the temperature of the assay that is equal to a greater than the viscosity of M9 medium, as measured by a suitable technique, such as an Ubbelohde, Ostwald and/or Brookfield viscosimeter).

Generally, suitable media for growing/maintaining nematode worms will be clear to the skilled person, and include for example the media generally used in the art, such as M9, S-buffer, and/or the further media described in the applications and handbooks mentioned hereinabove.

Preferably, the assays of the invention are based on the dauer phenotype as a biological read out, e.g. the entry into, the bypass of and/or the rescue from the dauer state, and/or any other property which results from and/or is associated with the so-called dauer decision.

For instance, an assay based upon entry into/bypass of the dauer state may comprise the following steps:

a) providing a sample of nematode worms (preferably eggs, L1 or L2 worms, and most preferably L1 worms);
b) keeping said sample under conditions such, without the presence of any compound(s) to be tested, at least 50%, and preferably at least 60 %, and more preferably at least 70 %, even more preferably at least 80 %, such as 85-100% of the nematodes present in said sample would enter the dauer state (at least during the time used for the assay, such as at least 1 day, for example 2-4 days - e.g. about 72 hours - as further described below);

c) exposing the sample to the compound(s) to be tested;

d) measuring either the number of worms that enter the dauer state, and/or measuring the number of worms that grow into adults.

Preferably, in such an assay, the conditions used in step b) are such that, in the presence of a reference compound (such as a vanadate compound, e.g. sodium orthovanadate) at a suitable concentration (such as between 0.5 and 2 milliMolar, which is particularly suited for vanadate), the amount of worms that enter the dauer state is at least 10% less (i.e. lower in absolute difference of percentages as also referred to above), preferably at least 20% less, more preferably at least 30% less, than the amount of worms that enter the dauer state without the presence of any such reference compound(at least during the time used for the assay, such as at least 1 day, for example 2-4 days - e.g. about 72 hours - as further described below).

For instance, the conditions used in step b) may be such that, in the presence of a reference compound (such as a vanadate compound, e.g. sodium orthovanadate) at a suitable concentration (such as between 0.5 and 2 milliMolar, which is particularly suited for vanadate), the amount of worms that enter the dauer state is less than 50%, preferably less than 40%, even more preferably less than 30% (at least during the time used for the assay, such as at least 1 day, for example 2-4 days - e.g. about 72 hours - as further described below, and depending on the amount of worms that would enter the dauer state without the

presence of the reference), although the invention in its broadest sense is not limited thereto.

An assay based upon rescue from the dauer state may comprise the following steps:

a) providing a sample of nematode worms in the dauer state;
b) keeping said sample under conditions such that, without the presence of any compound to be tested, least 50%, and preferably at least 60 %, and more preferably at least 70 %, even more preferably at least 80 %, such as 85-100% of the nematodes present in said sample would remain in the dauer state (at least for the time of the assay, such as between 1 and 96 hrs, such as between 12 and 72 hours, such as about 24-48 hours);
c) exposing the sample to the compound(s) to be tested;
d) measuring either the number of worms that remain in the dauer state, and/or measuring the number of worms that go out of the dauer state (e.g. become adults).

Preferably, in such an assay, the conditions used in step b) are such that, in the presence of a reference compound (such as a vanadate compound, e.g. sodium orthovanadate) at a suitable concentration (such as between 0.5 and 2 milliMolar, which is particularly suited for vanadate), the amount of worms that remain in the dauer state is at least 10% less (i.e. lower in absolute difference of percentages as also referred to above), preferably at least 20% less,

more preferably at least 30% less, than the amount of worms that remain in the dauer state without the presence of any such reference compound (at least during the time used for the assay, such as between 1 and 96 hrs, such as between 12 and 72 hours, such as about 24-48 hours).

For instance, the conditions used in step b) may be such that, (such as a vanadate compound, e.g. sodium orthovanadate) at a suitable concentration (such as between 0.5 and 2 milliMolar, which is particularly suited for vanadate), the amount of worms that remain in the dauer state is less than 50%, preferably less than 40%, even more preferably less than 30% (at least during the time used for the assay, such as between 1 and 96 hrs, such as between 12 and 72 hours, such as about 24-48 hours, and depending on the amount of worms that would remain in the dauer state without the presence of the reference), although the invention in its broadest sense is not limited thereto.

Techniques for distinguishing, in a sample, and preferably in an automated and/or multi-well plate format, the number of adults and/or the number of dauers will be clear to the skilled person and may include visual/manual techniques, and/or the non-visual detection techniques described in the applications referred to above.

In the assays of the invention, each individual sample of nematode worms will generally be exposed to a single compound to be tested, at a single

concentration; with different samples (e.g. as present in the different wells of the multi-well plate used) being exposed either to different concentrations of the same compound (e.g. to establish a dose response curve for said compound), to one or more different compounds (which may for instance be part of a chemical library and/or of a chemical class or series, such as a series of closely related structural analogues), or both (e.g. to the same and/or different compounds at different concentrations).

It is also within the scope of the invention to expose the (sample of) nematodes to two or more compounds - at essentially the same time or sequentially (e.g. with an intermediate washing step) - for example to determine whether the two compounds have an effect which is the same or different from both the compounds separately (e.g. to provide a synergistic effect or an inhibitory or competitive effect).

Furthermore, it is within the scope of the invention to use one or more reference samples, e.g. samples without any compound(s) present, and/or with a predetermined amount of a reference compound. The invention also includes the use, in an assay, of two or more samples of nematode worms of different strains, e.g. to compare (the effect of the compound(s) to be tested on) the different strains, in which said different strains may also be reference strains, such as wildtype, N2 or Hawaiian.

In a preferred embodiment, an assay based on dauer entry/bypass is carried out in a multiwell plate format, under the following conditions:

- use of a sample of between 2 and 100, preferably between 10 and 80, more preferably between 15 and 60 worms, such as 20 or 50 worms, preferably eggs, L1 or L2, most preferably L1.
- a temperature of between 10°C and 30 °C, preferably between 20°C and 27 °C, such as 21, 22, 23, 24, 25 or 26°C, depending on the specific strain used.
  For example, for DR1564: *daf-2(m41)*, usually a temperature of about 21, 22, 23, 24 °C will be preferred, with a temperature of between 21 and 22°C being particularly preferred.
  For CB1368: *daf-2(e1368)*, usually a temperature of 24, 25 or 26°C will be preferred, with 25°C being particularly preferred.
- a concentration of the compound(s) to be tested of between 0.1 nanomolar and 100 milimolar, preferably between 1 nanomolar and 10 milimolar, more preferably between 1 micromolar and 200 micromolar, such as about 20 micromolar. The compound may be taken up by the nematodes in any suitable manner, such as by drinking, soaking, via the gastrointestinal tract (e.g. the gut), via the cuticle (e.g. by diffusion or an active transport mechanism), and/or via openings in the cuticle, such as amphid sensory neurons. Generally, the compound will be mixed with or otherwise incorporated into the medium used;
- a time of contact with the compound(s) to be tested of between 0.1 minute and 100 hours, preferably between 1 minute and 90 hours, such as

about 1 hour to 72 hours. For instance, the sample of nematodes may be contacted with the compound(s) to be tested for only a brief period of time, e.g. between 1 minute and 2 hours, such as between 20 minutes and 1.5 hours, upon which the sample of nematodes may be washed and further cultivated on fresh medium (i.e. without compound), or the sample of nematodes may be contacted with the compound(s) to be tested for essentially the entire duration of the assay (e.g. for 1-3 days or more). For assays involving (the bypass of) dauer formation (e.g. starting from L1), the time of contact will generally encompass two or mores stages of development, and most preferably be between 1 and 4 days, such as about 2-3 days (e.g. 48 to 72 hours).

- a (total) time of incubation of the sample of between 0.1 minute and 100 hours, preferably between 1 minute and 90 hours, such as about 1 hour to 72 hours. For assays involving dauer entry/bypass (e.g. starting from L1), the total incubation time will generally encompass two or mores stages of development, and most preferably be between 1 and 4 days, such as about 2-3 days (e.g. 48 to 72 hours);
- the use of a liquid or viscous medium (in which viscous is as defined above), such as S-buffer, M9 or one of the other media referred to in the patent applications mentioned above (as referred to above), with S-buffer being particularly preferred.
- The presence of a suitable source of food - for example bacteria such as *E. coli* - in a suitable

amount, e.g. between 0.001 and 10 % (w/v), preferably between 0.01 and 1%, more preferably between 0.1 and 0.2 %, such as about 0.125 % w/v, based on the total medium.

Conditions for assays based on dauer rescue are further described below and/or in PCT US 98/10800 and US-A-6,225,120.

Although the conditions described above are particularly preferred, more generally, according to the invention, the nematode strains with increased sensitivity of the insulin signalling pathway (as further defined above) may be used with advantage in any *C. elegans*-based assay technique involving and/or relating to insulin-signalling, insulin signal transduction, biological responses to insulin and/or insulin-type compounds, and/or the insulin pathway. These assays may be based on any suitable phenotypical read out, including but not limited to dauer entry, bypass and/or rescue as described above.

Therefore, in accordance with one aspect of the invention, there is provided a method for the identification of a compound which is capable of modulating insulin signalling pathways, which method comprises:

providing *C. elegans* larvae of a strain of sensitized genetic background to the insulin signalling pathway;

contacting said larvae with a test compound in growth favouring conditions, i.e. including food; and

screening for growth to adulthood, i.e. bypass of or release from the dauer larval state.

A "sensitized genetic background" may be defined

herein by comparison to the reference *daf-2* allele, *e1370* (Figure 2 is a print of the acedb database entry on *daf-2*). The term "sensitized genetic background" encompasses *C. elegans* strains which exhibits greater sensitivity to test compounds than the *daf-2(e1370)* allele.

The method of the invention is suitable for use with essentially any *C. elegans* strain which exhibits a dauer phenotype as a result of defect, for example a mutation, in a gene encoding a component of the insulin signalling pathway or other intervention affecting the insulin signalling pathway and which exhibits a "sensitized genetic background" as compared to the *daf-2(e1370)* mutant.

In a preferred embodiment the method of the invention may be carried out using *C. elegans* strain DR1564 containing the *daf-2(m41)* mutation which exhibit a dauer-constitutive phenotype. Use of strains carrying this allele in compound screens based on bypass of/rescue from dauer is illustrated in the accompanying Examples. Table 6 compares the activity of 94 compounds, which were found to be positive in a primary screen of 8,000 compounds using DR1564: *daf-2(m41)*, as part of Example 1, in a retest on the *m41* allele bearing strain DR1564 and on the *daf-2* alleles bearing strains CB1368: *daf-2(e1368)* and *daf-2(e1370)*. DR1564: *daf-2(m41)* was found to be more sensitive to compound activities than CB1368: *daf-2(e1368)*, with 56% and 27% confirmation rate, respectively. The strain CB1370 containing the *daf-2* reference allele *e1370* could not be rescued by any of the 94 compounds.

Other sensitized backgrounds in addition to *daf-2(m41)* may be used in accordance with the invention. Since both *m41* and *e1368* belong to class I alleles in the classification of Gems et al. 1998, Genetics 150: 129-155, while *e1370* belongs to class II, it is likely that other class I alleles are also useful as sensitized genetic background. Typically class I alleles are mutations in the ligand binding domain, and class II mutations are located in the kinase domain. The precise molecular lesion of *m41* is unknown.

Other *C. elegans* strains with sensitized genetic backgrounds which may be used in accordance with the invention include strains exhibiting a dauer phenotype which comprise loss of function or reduction of function mutations in genes downstream of the insulin receptor (*daf-2*). A particular example is the *age-1* mutation, a mutation in the catalytic subunit of the PI3-kinase (see Figure 1 and table 1). While gain of function alleles of *akt-1* or *pdk-1* are not able to rescue *daf-2*(*e1370*), they do rescue *age-1* mutations (Paradis and Ruvkun 1998, Genes & Dev 12:2488-2489, Paradis and Ruvkun 1999, Genes & Dev 13:1438-1452).

While there are no mutations known in the regulatory subunit of the PI3-kinase (located on the yac clones Y119C1 and Y110A7), knock-out mutations in these genes may be generated by methods known by the art (Zwaal et al. 1993, PNAS 90: 7431-35; Liu et al. 1999, Genome Research 9:859-867). Other suitable strains carry loss of function mutations in the genes encoding AKT protein kinases. Since there are two redundantly acting AKT potein kinases (Paradis and

Ruvkun 1998, Genes & Dev 12:2488-2489), a double mutation of knock-outs of both *akt-1* and *akt-2* may be to be constructed by simple crossing. Another potential useful mutation is the loss of function mutation in *pdk-1(sa680)*, as described in Paradis and Ruvkun 1999, above cit.

In a further embodiment of the method of the invention, a *C. elegans* strain having a sensitized genetic background may be obtained by inhibiting proteins of the insulin-receptor pathway using specific inhibitor compounds. In particular, inhibitors of the PI3-kinase are known, such as Wortmannin and LY294002. Barbar et al. 1999, Neurobiol Aging 20:513-519 demonstrate the activity of LY294002 in inducing dauer formation. The inventors own experiments also illustrate the activity of Wortmannin (Figure 4).

RNAi inhibition is still another method of generating *C. elegans* strains with loss of function phenotypes suitable for use in the method of the invention. Methods of inhibiting expression of specific genes in *C. elegans* using RNAi are well known in the art and described, for example by Fire et al., Nature 391:801-811 (1998); Timmins and Fire, Nature 395:854 (1998) and Plaetinck et al., WO 00/01846. Most preferred are the techniques described in WO 00/01846 which use special bacterial strains as food source to obtain double stranded RNA inhibition.

In yet another embodiment of the present invention, sensitized strains may be used which comprise gain of function mutations of *daf-18* or *daf-16* or of the *C. elegans* homologs of PTP-1B or

SHIP2. Generation of gain of function mutations of serine or threonine phosphorylation sites, as disclosed for *daf-16* by Paradis and Ruvkun 1998, above cit., and by Kops et al. 1999, Nature 398: 630-634, is straightforward for researchers experienced in the state of the art, as demonstrated by Nakae et al. 2000, EMBO 19: 989-996 for FKHR, a human homologue of *daf-16*.

Yet another sensitized genetic background may be derived by using mutants defective in perception of environmental signals that regulate insulin signalling, such as pheromone, food and temperature signals, or mutations in the neural processing of said signals, or mutations in the secretion of insulin-like molecules or in one of the genes encoding for an insulin-like molecule. In a preferred embodiment *tph-1*(*mg280*) is used, a mutant deficient in tryptophan hydroxylase, necessary for serotonin biosynthesis. *C. elegans* worms with this mutation accumulate large stores of fat and to some extend form dauer larvae because of inability to process the food sensation, together with impaired temperature sensation (Sze et al. 2000, Nature 403: 560-564). Other suitable sensitized genetic backgrounds comprise daf-c mutations in *daf-1, daf-4, daf-7, daf-8, daf-11, daf-14, daf-21, daf-19* or *daf-28*. Furthermore, dominant activation mutations in neuronal G proteins, as described by Zwaal et al. 1997, Genetics 145: 715-727, may also serve as sensitized background.

Several synthetic dauer forming mutations are known, which enhance other genetic backgrounds to form dauer mutations. One specific example, the double

unc-64(e246); unc-31(e928), is given by Ailion et al. 1999, PNAS 96, 7394-7397. Since *unc-64* encodes for a homolog of syntaxin, a protein involved in synaptic transmission and other types of Ca^{2+}-reulated secretion and *unc-31* encodes for a homolog of CAPS, Ca^{2+}-dependent activator protein for secretion and insulin release in pancreatic ß cells is determined by Ca^{2+}-regulated secretion the simplest model is that, the Daf-c phenotype of the double mutation is caused by a shut down of release of either insulin like molecules themselves or of neurotransmitters that stimulate insulin release (Ailion et al. 1999, PNAS 96, 7394-7397).

Sensitized worm strains which comprise any combination of two or more synthetic dauer formation mutations amongst each other, or in combination with dauer constitutive mutations, as examples are provided above, or any combination of dauer constiutive mutations with each other may be used in the method of the invention. An example can be drawn from Ogg et al. 1997, Nature 389: 994-999, where a *daf-2; daf-1* double mutant induces dauer formation at temperatures far below temperatures necessary for each of the single mutation to induce dauer formation.

The disclosed screening method is based on bypass of/release from the dauer larval state. There are several different ways in which to screen for bypass of/release from the dauer state which may be used in accordance with the invention, as described below. Furthermore, it is possible to use phenotypes of Daf genes other than dauer, including but limited to, fat storage, regulation of metabolic enzymes or

stress resistance pathways or any other biochemically, transcriptionally or posttranscriptionally regulated effect that is measurable as the basis of an assay read-out in accordance with the invention.

In accordance with a second aspect the invention also provides a method for the identification of a compound which is capable of modulating insulin signalling pathways, which method comprises:

providing *C. elegans* larvae of a strain of sensitized genetic background to the insulin signalling pathway;

contacting said larvae with a test compound in growth favouring conditions, i.e. including food; and

screening for growth to adulthood, i.e. bypass of or release from the dauer larval state, wherein conditions of assay are selected such that a basal level of bypass of or release from the dauer larval state is observed in the absence of the test compound.

The second aspect of the present invention comprises of a sensitized assay condition, in contrary to tight screening conditions usually performed in screens to isolate genetic suppressors of *daf-2*, e.g. *daf-16* alleles (Riddle et al. 1981, Nature 290:668-671; Gottlieb & Ruvkun 1994, Genetics 137: 107-120).

The inventors provide a method of setting the assay conditions in way that a basal level of release from the dauer larval state is already present in controls. The basal level of release from the dauer larval state may for example be measured by counting the number of worms growing beyond the dauer stage in

a sufficiently large number of control wells (containing the solvent alone but no test compounds). The basal level of release from the dauer larval state will preferably be between 0.1% and 60% rescue, more preferably between 1% and 50% rescue and most preferably between 2% and 40% rescue, such as 10% to 20% rescue. While the minimal number of growing worms or residual activity is derived from sensitizing the assay conditions, the maximal number is derived from experience to optimise signal to noise ratio.

Although in a preferred embodiment the method of the invention uses the temperature sensitivity of *daf-2* mutations, such as *m41*, to sensitize assay conditions, any set of conditions that sensitize the assay over the strict genetic screen conditions is within the scope of the invention, in particular conditions that show growth between 0.1% and 60%, preferentially between 1% and 50%, most preferentially between 2% and 40%, such as 10% to 20%, in cases where the readout of the assay is related to bypass of or release from the dauer-constitutive phenotype.

Another embodiment of the invention uses genetic means to sensitize assay conditions to the desired basal level of release from the dauer larval state. For example Ogg & Ruvkun (1998), Mol. Cell 2: 887-893, disclose a double mutation *daf-2; daf-18*, which gives rescue (L4 and adults) at a level of 2.2%. In addition, mutations known as Daf-d for dauer defective, especially weak mutations, can be used in the present invention. Also gain of function mutations, as there are known *pdk-1(mg142)*, (Paradis and Ruvkun 1999, Genes & Dev 13:1438-1452) and

akt-1(mg144), (Paradis and Ruvkun 1998, Genes & Dev 12:2488-2489), can be used to rescue from dauer formation to a certain percentage. Furthermore, gain of function, in particular at phosphorylation sites, or loss of function mutations can be generated by methods known in the art (see citations in the section further above).

Also suitable for use in the method of the invention are *C. elegans* strains which comprise a mutation in a gene downstream of the insulin receptor in the insulin signalling pathway which leads to a reduction in the function of the product of the mutated gene but not a complete loss of function. Residual activity of the product encoded by the gene mutated in such strains may be sufficient to confer a basal level of release from the dauer larval state.

Another embodiment of the invention comprises the incomplete loss of function typically seen with RNAi experiments. Since the disclosed methods rely on growth of worms in presence of *E. coli*, methods of obtaining RNA inhibition via feeding of appropriately engineered bacterial strains may be used as discribed in Plaetinck et al., WO 00/01846.

Still another embodiment of the invention comprises incomplete rescue typically obtained by heterologous transgenes. For example, a strain *daf-16; daf-2; Ex[daf-16b::hsFKHR]* has been constructed in which *daf-16* loss of function, in itself rescuing from *daf-2* induced dauer formation, is rescued by the human homolog FKHR under the *C. elegans* daf-16b promoter. This rescue is incomplete, to about 60% dauer formation, so that 40% grow to adulthood

(Gary Ruvkun, personal communication). Any other homologue of *daf-16*, for example the human genes FKHRL1 or AFX, or others, mammalian or human, could be used in combination of suitable promoters, either one of the endogenous *daf-16* promoters, daf-16a or daf-16b or both, or a heterologous promoter, preferably with ubiquitous expression or nervous system expression.

Still another embodiment of the invention is based on the addition of pheromone preparations so that the fraction of worms growing adults is driven below 60%, preferably below 40%, more preferably below 40%, such as between 10% and 20%. As already mentioned, Sze and co-workers (Nature 403: 560-564) generated a *tph-1(mg280)* mutation, which induces dauer arrest at 15%, mimicking low food supply and with some resistance to temperature control. However, since the dauer arrest can be enhanced to 80% using a *daf-7* mutation, which are defective in production of a TGFß like molecule signalling the absence of pheromone, addition of pheromone could achieve the desired level of 80% dauer formation as an alternative to the double mutant. Pheromone preparations may be obtained after the method of Golden & Riddle 1984, PNAS 81: 819-823.

This screening method of the invention is again based on bypass of/release from the dauer larval state and there are several different ways of screening for bypass of/release from dauer which may be used in accordance with the invention, see below. The invention can as well be based on any other phenotype relating to the insulin pathway, such as are observed in *daf-2* mutations, including but not exclusive to fat storage, regulation of metabolic enzymes or stress

resistance pathways or any other biochemically, transcriptionally or posttranscriptionally regulated effect that is measurable.

Set out below are ways of screening for bypass of or release from the dauer larval state which may be used in accordance with the invention.

One of the simplest and most exact methods of measuring bypass of/rescue from dauer larvae formation is counting of adults. Counting of adults may be achieved using automated means, e.g. automatic plate readers, allowing the screen to be performed in mid-to-high throughput format in multiwell microtiter plates.

A further method of screening for bypass of or rescue from the dauer phenotype exemplified herein is based on staining of adults using Nile Red an automated data acquisition (Example 2). Other methods of screening for release from the dauer larval state are also encompassed by the invention.

As an alternative to direct counting of adults indirect measurements, for example the consumption of food by measuring turbidity, may form a usable readout.

Further methods of screening for bypass of/release from the dauer larval state are based on the use of reporter transgene. Suitable reporter transgene constructs generally comprise a promoter or promoter fragment operably linked to a reporter gene. The promoter or promoter fragment is one which is capable of directing strong gene expression in adult

C. elegans but no or weak gene expression in dauer larvae, such as a promoter which is regulated by the *daf-2* signalling pathway (e.g. promoters regulated by the transcription factor *daf-16*) or vice versa (i.e. no or weak expression in adult, strong expression in dauer larvae. The term "operably linked" refers to a juxtaposition in which both components function in their intended manner, i.e. the promoter drives expression of the reporter gene. One example of a suitable transgene is a construct comprising the *C. elegans vit-2* promoter operably linked to a luciferase reporter gene. Any other promoter that shows strong expression in adults but no or weak expression in dauer larvae may be used as an alternative to the *vit-2* promoter. Other reporter genes may be used as alternatives to luciferase. Preferably the reporter gene will be one encoding a product which is directly or indirectly detectable in the worm, for example a fluorescent, luminescent or coloured product, e.g. GFP or lacZ. Preferably expression of the reporter gene product in the worm will be measurable using an automated plate reader.

The inventors provide methods for constructing ctl-1::luciferase and a sod-3::luciferase reporter transgenes, the *ctl-1* and *sod-3* genes encoding respective a cytosolic catalase with markedly increase expression in *daf-2* dauer larvae (Taub et al. 1999, Nature 399:162-166) and a manganese superoxide dismutase strongly up-regulated in *daf-2* mutant adults (Honda and Honda 1999, FASEB 13: 1385-1393). The regulation of a mitochondrial manganese superoxide dismutase by *daf-2* is of particular interest, since it

has recently been shown that overexpression of a Mn-SOD in vascular endothelial cells can suppress several pathways involved in hyperglycaemic damage, indicating that those damages are caused by production of superoxides (Nishikawa et al. 2000, Nature 404: 787-790).

To perform a screen using a reporter transgene the transgene must first be introduced into the *C. elegans* used in the screen. This may be achieved using standard techniques for the construction of transgenic *C. elegans* well known in the art and described, for example, in Methods in Cell Biology, Vol 48, Ed. H.F.Epstein and D.C.Shakes, Academic Press.

Table 1: targets of the insulin receptor pathway

Targets	**Human homologs**	**Function**	**Validation**	**Desired intervent ion**
DAF-2	IR	Receptor tyrosin kinase	*e1391* equals het. mutation of an morbidly obese diabetic patient	+
	PTP-1B	Protein tyrosin phosphatase	Mouse k.o. insulin hypersensitive	B
DAF-2	IRS-1, -2	Insulin receptor substrate	IR/+; IRS-1/+ age onset diabetes, IRS2 diabetic	+
AGE-1	p110	PI3-kinase catalytic subunit	p110β insulin responsive	+
	p85/p55	PI3-kinase regulatory subunit	p85α k.o. insulin hypersensitive	+/B
DAF-18	PTEN	PI-3' phosphatase	maternal and zygotic minus rescues *daf-2(e1370)*	B
	SHIP2	PI-5' phosphatase	Overexpression inhibits AKT activation	B
PDK-1	PDK1	AKT phosphorylation	gf rescues dauers, lf induces dauers	+
AKT-1, AKT-2	AKT =PKB	Forkhead TF phosphorylation	gf rescues, double RNAi induce dauers	+
DAF-16	FKHR, FKHRL1	Transkription factor	lf rescues *daf-2 (e1370)*	B

The present invention will be further understood with reference to the following Experimental examples, together with the accompanying Figures in which:

Figure 1 illustrates the insulin receptor signalling pathway of *C. elegans*.

Figure 2 is a print of the acedb database entry on *daf-2*.

Figure 3 is a graph to show that vanadates can rescue the genetic insulin resistance caused by *daf-2* mutations in *C. elegans* in an assay based on bypass of/rescue from the dauer larval state.

Figure 4 is a graph to show that wortmannin further enhances insulin resistance caused by *daf-2* mutations in *C. elegans* in an assay based on bypass of/rescue from the dauer larval state.

Figure 5 scatter plot of mean and variance of controls for the screening experiment described in Example 1 (a) screening, (b) DRC.

Figure 6 shows distribution of controls and a maximum likelihood of fit of a negative binomial distribution for data generated in the screening experiment described in Example 1.

Figure 7 shows distribution of controls in % of the average of the plate for data generated in the screening experiment described in Example 1.

Figure 8 shows the results of a representative nile red staining experiment (Example 2).

Figure 9 is a representation of pGQ1.

Figure 10 is a representation of pDW2020.

Figure 11 shows the complete nucleotide sequence of pDW2020.

Figure 12 shows the complete nucleotide sequence of pGQ1.

Figure 13 is a print of the acedb database entry on *ctl-1*.

Figure 14 is a representation of pGQ2.

Figure 15 is a representation of pCluc6.

Figure 16 shows the complete nucleotide sequence of pCluc6.

Figure 17 shows the complete nucleotide sequence of pGQ2.

Figure 18 is a print of the acedb database entry on *sod-3*.

Figure 19 is a representation of pGQ3.

Figure 20 shows the complete nucleotide sequence of pGQ3.

Figure 21 is a representation of pGQ4.

Figure 22 shows the complete nucleotide sequence of pGQ4.

Figure 23 illustrates the cloning of pCluc6.

Example 1: screening 23,040 compounds for activity in the insulin-receptor pathway.

Materials used

- 9cm plates seeded with OP50,
- three weeks old stock plates of daf-2(m41)
- M9 buffer
- S-complete buffer
- 96-well plates flat bottom NUCLON Surface
- 96-well plates U-bottom for dilutions compounds
- HB101 bacteria (routinely available)
- compounds (80 per 96-well plates) 10mM concentration in 100% DMSO

Method

Test of the batch of bacteria to be used as food:

- Growth of HB101

- fill a 2 liter Erlenmeyer sterile with 0,51 DYT medium
- inoculate with *E-coli* HB101 single colony
- let shake for 24 hours at 250 rpm and 37 C
- centrifuge in sterile 250ml centrifuge tubes 10 min 10000rpm.
- resuspend in 120 ml S-basal medium (pipette up and down and shake)
- transfer to 8 15ml falcon tubes that were weighed in advance
- centrifuge second time 10 min 6000rpm
- weigh the pellet
- store at 4 C

- Test of the batch:

- chunk a couple of plates of *m41*
- bleach plates after 4 days, let eggs hatch on unseeded plate at 15 C
- wash off L1's after one night
- bring 50 L1 in 80 µl S-complete in one 96 well plate
- add 10 µl 2% DMSO
- add 10µl of 1.25% of the batch of bacteria to be tested
- put plate in closed box in the 21 C incubator
- check on number of dauers after three days of growth, should be no more then 10
- if the batch is approved, it can be stored undiluted at 4 C for several weeks

Protocol

Thursday:

- chunk 9 cm plates (take 1 plate/96-well plate to be filled)
- grow in middle incubator at 15 C (preferably same shelf)

Monday : bleach plates

- wash off in M9
- 10 plates/falcon 15ml
- put washed off plates back in 15 C incubator (only uncontaminated ones)
- spin down at 1300rpm/3min
- suck off M9
- add bleach
- when most worms are broken, add sucrose, shake, add 2 ml M9
- spin at 1300rpm/3 min
- carefully remove eggs from bottom of layer of M9, bring in new falcon
- add M9 to 15ml
- spin down 1300rpm/3min
- add M9
- spin down 1300rpm/3min
- suck away M9 to 1ml
- divide eggs from one falcon over 3 unseeded plates
- put plates at 15 C to let eggs hatch

Tuesday :

a) preparation of the compound-plates

- dilute aliquot of compound in 96-well plate to 200μM in S-buffer (DMSO conc. 2%).
- replicate plates: four plates 10μl 200μM compound per well
- write number and replicate number on plates
- if there was no DMSO in col 1 and 12 of the aliquoted plate it has to be added (add 11μl of 2% DMSO)
- write number of the plate and the replicate on the lid of the plates

b) preparation of the worms solution

1) "bleached L1's"

- wash L1 off plates in S-complete, 4 plates/15ml falcon
- spin down at 1300rpm/3min
- add fresh S-complete to 100ml
- count worms in 10 μl
- keep worm suspension at 15 C while counting
- dilute further to approximately 50 worms/80 μl, count again
- mix well

2) "washed L1's"

- wash off plates that were washed yesterday
- spin down (1300rpm/3min), add S-complete, wash twice
- filter suspension over 11 micron mesh over embroidery hoop into lid of 9cm plate
- wash L1's one more time
- dilute to 50 worms/80μl in the same way as bleached L1

c) Final steps:

- add 1.25% freshly diluted HB101 bacteria to worm suspension so that final concentration is 0.125% (1 volume of bacteria to 8 of worms)
- add 90 µl of worm-bacteria suspension/well with electronic pipette
- put plates in closed boxes with wet tissues in 21°C incubator
- monitor temperature in control box in incubator while growing (try to put boxes at the same shelf, avoid contact of the boxes to metal of cooling device!)

Friday: Scoring:

1. count 8 negative control wells/plate
2. plot the average and variance of the negative controls from each plate
3. check for differences between boxes, differently treated L1's and replicates
4. if necessary define several groups, remove outliers
5. make a distribution of the negative controls per group (plot # of wells to the number of worms/well)
6. for each defined group: fit a negative binomial distribution to the negative controls and determine the number of adults for a cut-off confidentiality of about 1% and about 0.1% (both sides for screen of dauer rescue and dauer enhancers)

7. screening for dauer rescue is possible if average of negative control is between 0 and 15 adults/well, screening for dauer enhancers is possible if the average is above 5
8. screen through the plates and count the wells with high number of adults
9. if the number of adults in the well is below the cut-off value leave it
10. if the number of adults is above or at the 1% cut-off value circle the well as positive (for each of the replicate with a different color) and write the number in the circle
11. if the number of adults is above the 0.1% cut-off value estimate the number of adults
12. Put the lids of the 4 replicates of the same plate on top of each other
13. Search for wells with 2 or more positives in the 4 (or 3) replicates
14. Write down the number of the adults of each of the 4 (or 3) replicates

Robustness

While the controls active in the pathway show the sensitivity of the assay (see Figures 2 and 3), its specificity is determined by testing a range of compounds outside the pathway. Together with the reference compounds acting in the insulin signalling pathway, of which only Wortmannin and vanadates were active, anti-diabetics with a mode of action outside the insulin pathway, including 3 guanidine derivatives (acting on glucose uptake and metabolism), 5 PPARγ ligands (stimulating adipocyte differentiation) and 6

sulphonylureas (which act by increasing insulin secretion) were tested. None was found to be active in the assay. Further confirmation of the specificity of the screen is derived from testing a library of 800 compounds from Tocris-Cookson, containing mainly neurological actives, at 20 µM in triplicates. Only 4 compounds rescued dauer formation, a rate not higher than for random libraries (see results).

Table 2

Name of compound	**supply**	**MW**	**drug class/ disease area/ action(s)**	**solvent**	Concentrations tested in µM- (lethal) **rescue,** ***dauer enhancer***
Synthalin	ICN	354.5	guanidine derivative, also NMDA antagonist	DMSO	(333; 166.7; 83.3; 33.3); 20; 16.6; 8.3; 3.3
Metformin HCl (1,1-dimethylbiguanide)	Sigma	165.6	guanidine derivative, biguanides, MOA?: decrease hepatic glucose production	DMSO	333; 166.7; 83.3; 33.3; 20
Phenformin HCl (phenethylbiguanide)	Sigma	241.7	guanidine derivative, biguanides, MOA?: decrease hepatic glucose production	DMSO	333; 166.7; 83.3; 33.3; 20
HNMPA(AM)3	Calbiochem	454.4	insulin receptor tyrosine kinase inhibitor	DMSO	20
Rapamycin	ICN	914.2	insulin signalling enhancer, inhibitor of the mammalian target of rapamycin (mTOR) which is a downstream target of Akt and implicated in Akt's negative regulation of insulin signalling i.e.	DMSO	33.3; 16.6; 8.3;

			serine/threonine phosphorylation of IRS-1		
Quercetin	Sigma	338.3	insulin signalling inhibitor, inhibitor of phosphatidylinositol 3-kinase and of several other ATP-requiring enzymes e.g. PI4K, PKC, EGFR, calcium, SERCA activator by interacting with nucleotide binding site to mask PLB inhibition	DMSO	20
okadaic acid	Calbioc hem	805	insulin signalling inhibitor, inhibits PP2A and PP1	DMSO	10; 5; 2.5; 0.6
PD 98059	Calbioc hem	267.3	insulin signalling inhibitor, MEK1 inhibitor	DMSO	20
Wortmannin	***Sigma***	***428.4***	***insulin signalling inhibitor, phosphatidylinositol 3-kinase inhibitor (potent and specific), inhibitor of neutrophil activation and of FMLP-mediated phospholipase D activation***	***DMSO***	***20***
LY 294002	Sigma	307.3	insulin signalling inhibitor, phosphatidylinositol 3-kinase inhibitor (specific)	DMSO	100, 20
phorbol 12-myristate 13-acetate (PMA)	Biomol	616.8	insulin signalling inhibitor, PKC activator (elicits serine/threonine phosphorylation of IRS-1)	DMSO	20
Phosphatidylinositol-3,4,5-trisphosphate [stearyl, arachidonoyl, tetraammonium salt)	Alexis	1123.1	insulin signalling, identical to endogenous PI(3,4,5)P3 (not an analog containing only saturated fatty acid residues, therefore greater biological activity), activates Ca2+-insensitive PKC, activates Akt (a serine/threonine kinase) by directly interacting with the Akt pleckstrin homology (PH) domain	DMSO	2.8; 1.4; 0.7

Phosphatidylinositol-3,4-bisphosphate [L-alpha-] (dipalmitoyl, pentaammonium salt)	Calbiochem	1056.2	insulin signalling, mimics endogenous PI(3,4)P2, activates Ca2+-insensitive PKC, activates Akt (a serine/threonine kinase) by directly interacting with the Akt pleckstrin homology (PH) domain	DMSO	3.17; 1.9; 1.58; 0.79
Phosphatidylinositol-3,4,5-trisphosphate [L-alpha-] (dipalmitoyl, heptaammonium salt)	Calbiochem	1170.2	insulin signalling, mimics endogenous PI(3,4,5)P3, activates Ca2+-insensitive PKC, activates Akt (a serine/threonine kinase) by directly interacting with the Akt pleckstrin homology (PH) domain	DMSO	2.96; 1.74; 1.48
Thalidomide	ICN	258.2	insulin signalling, TNF inhibitor	DMSO	333; 166.7; 83.3; 33.3; 20
Perhexiline	Sigma	393.6	insulin, carbohydrate metabolism, inhibitor of myocardial carnitine palmitoyltransferase-1 ("antidiabetics"), sodium, calcium, dual Na+/Ca2+ (T-type) channel blocker, anti-angina (coronary vasodilator), diuretic	DMSO	(333; 166.7; 83.3; 33.3); 20; 16.6; 8.3; 3.3
L-arginine	Sigma	174.2	nitric oxide, insulin secretagogue (NO dependent)	water	333; 166.7; 83.3; 33.3; 20
D-arginine	Sigma	174.2	nitric oxide, negative control of L-arginine (insulin secretagogue)	water	20
LY 171883	Sigma	318.4	PPARgamma activator (weak), selective LTD4 antagonist	DMSO	20
linoleic acid (9,12-octadecadienoic acid)	Sigma	280.4	PPARgamma ligand	DMSO	(333; 166.7; 83.3; 33.3); 20; 16.6; 8.3; 3.3
Linolenic acid (9,12,15-octadecatrienoic acid)	Sigma	278.4	PPARgamma ligand	DMSO	(333; 166.7; 83.3; 33.3); 20; 16.6; 8.3; 3.3
Eicosatetraynoic acid [5,8,11,14-] (ETYA)	ICN	296.5	PPARgamma ligand, insulin sensitizers, eicosanoid	DMSO	333; 166.7; 83.3; 33.3; 20

Rosiglitazone (BRL49653)		359	PPARgamma-specific agonist (insulin-sensitizing properties, used in type II diabetes)	water	909; 500; 263; 135; 55; 27.6; 13.85
Chelerythrine chloride	Sigma	383.8	protein kinase C inhibitor (potent, selective, IC50 0.7μM)	DMSO	10
Cantharidic acid	Sigma	214.2	protein phosphatase 2A inhibitor (IC50 53 nM)	DMSO	20
Phenylarsine oxide	Calbiochem	168	PTP inhibitor, also inhibits PI3-kinase activity	DMSO	20
Bromotetramisole oxalate [L-p-]	Biomol	373.2	PTP inhibitor, also well known inhibitor of alkaline phosphatase, mimics the action of orthovanadate in the potentiation of fluorouracil antiproliferative activity	water	20
Bromotetramisole oxalate [D-p-]	Biomol	373.2	PTP inhibitor, also well known inhibitor of alkaline phosphatase, mimics the action of orthovanadate in the potentiation of fluorouracil antiproliferative activity: inactive isomer, negative control	water	20
Dephostatin	Calbiochem	168.2	PTP inhibitor, IC50 7.7μM, also nitric oxide donor (stable NO donor for S-nitrosation of proteins)	DMSO	333; 166.7; 83.3; 20
vanadium(II) chloride	Aldrich-Sigma	121.85	PTP inhibitor, vanadium compound	DMSO	20
vanadium(III) chloride	Aldrich-Sigma	157.3	PTP inhibitor, vanadium compound	DMSO	1000; 500; 250; 100; 20
vanadium(III) oxide	Aldrich-Sigma	149.88	PTP inhibitor, vanadium compound	DMSO	20
vanadium(IV) oxide	Aldrich-	165.88	PTP inhibitor, vanadium compound	DMSO	20

	Sigma				
vanadium(V) oxide	Aldrich-Sigma	181.88	PTP inhibitor, vanadium compound	DMSO	20
vanadyl sulfate	Aldrich-Sigma	163	PTP inhibitor, vanadium compound	DMSO	1000; 500; 250; 100; 20
vanadyl trifluoride	Fluka-Sigma	123.94	PTP inhibitor, vanadium compound	DMSO	20
mpV (Pic) (mono peroxo (picolinato) oxovanadate(V))	**Calbiochem**	**257.1**	**PTP inhibitor, vanadium compound**	**DMSO**	**1000; 500; 250;** 100; 20
sodium metavanadate	**Sigma**	**121.9**	**PTP inhibitor, vanadium compound, also inhibits ATPase and alkaline phosphatase**	**water**	**1000; 500; 250;** 100; 20
sodium orthovanadate	**Sigma**	**183.9**	**PTP inhibitor, vanadium compound, also inhibits ATPase and alkaline phosphatase**	**water**	**1000; 500; 250;** 100; 20
bpV (Phen) (Potassium Bisperoxo (1,10-phen anthroline) oxovanadate(V))	**Calbiochem**	**404.3**	**PTP inhibitor, vanadium compound, potent**	**DMSO**	**1000; 500; 250; 100; 20**
bpV(bipy) (potassium bisperoxo(bipyridine) oxovanadate(V)	Alexis	326.2	PTP inhibitor, vanadium compound, potent	DMSO	1000; 500; 250; 100; 20
bpV(Hopic) (di potassium bis peroxo(5-hydroxy pyridine-2-carboxyl)-oxovanadate(V))	**Alexis**	**347.2**	**PTP inhibitor, vanadium compound, potent**	**DMSO**	**1000; 500; 250; 100; 20**
bpV(pic)	**Alexis**	**367.3**	**PTP inhibitor, vanadium compound,**	**DMSO**	**1000; 500; 250;**

(dipotassium bisperoxo(picolinato)oxovanadate(V)			potent		100; 20
acetohexamide	ICN	324.4	sulfonylureas, first generation, MOA: insulin secretagogue by blocking K+(ATP) channels	DMSO	333; 166.7; 83.3; 33.3; 20
chlorpropamide	Sigma	276.7	sulfonylureas, first generation, MOA: insulin secretagogue by blocking K+(ATP) channels	DMSO	333; 166.7; 83.3; 33.3; 20
tolazamide	Sigma	311.4	sulfonylureas, first generation, MOA: insulin secretagogue by blocking K+(ATP) channels	DMSO	333; 166.7; 83.3; 33.3; 20
tolbutamide	Sigma	270.3	sulfonylureas, first generation, MOA: insulin secretagogue by blocking K+(ATP) channels	DMSO	333; 166.7; 83.3; 33.3; 20
glipizide	RBI	445.53	sulfonylureas, second generation, MOA: insulin secretagogue by blocking K+(ATP) channels	DMSO	333; 166.7; 83.3; 33.3; 20
glyburide (glybenclamide)	Tocris	494.1	sulfonylureas, second generation, MOA: insulin secretagogue by blocking K+(ATP) channels	DMSO	333; 166.7; 83.3; 33.3; 20
diazoxide	Tocris	230.7	potassium, K+ channel opener, avtivates ATP-sensitive K+ channels, antihypertensive, also stimulates K+ channels in pancreatic islet cells (prodiabetic side effects), diabetes	DMSO	333; 166.7; 83.3; 33.3; 20

Data aquisition

All screening was done at 20 µM compound concentration in quadruplicates, except 2000 compounds of Diverset in triplicates. Confirmation was done at 4 concentrations. Questionable dose responses were repeated, if necessary at lower concentrations and/or 2 fold dilution steps. All worms that bypassed dauer stage, L4s and adults, were counted under a Leica MZ12 dissection scope and together referred to as number of adults per well. First, the 8 negative controls (column 1) of all plates were counted, typically between 800 and 1280 (25 to 40 plates times 4 per screening session). Data were transferred to Excel files and average and variance of the 8 controls of each plate calculated and plotted.

Outliers of unusual high average or variance were removed for calculation, since they were found to have an inappropriately large effect on the calculations below (3 plates in the example of Figure 5a). Counting errors were found to have a rather weak effect. The number of wells was plotted against the number of adults per well and a negative binomial distribution fitted by maximum likelihood. In some cases it was necessary to split a session in two or three different subsessions mainly due to differences in incubator location or worm handling.

Then the number of adults per well where the cumulative negative binomial distribution was closest to 99% was determined and referred to as 1% cut-off. In the example shown in Figure 6, 20 adults per well

were at 1.10% indicating that the probability to have 20 or more adults per well is 1.10%. This calculates to a 4% chance for a single false positive in quadruplicates, but only to a 0.07% chance for a double false positive. Therefore a compound is positive, if at least 2 replicates have values at the cut-off or higher. In addition the 0.1% cut-off was determined similarly (24 adults in the example shown in Figure 6) and if at least 2 replicates were reaching that stronger value the compound was referred to as strong positive.

The plates were then screened through quickly to find wells with a high number adults, which were counted and if found to reach the cut-off value the position on the lid was circled and the exact value written in the circle. For higher numbers above the 0.1% cut-off an estimate rather than an exact count proved sufficient. Finally the transparent lids of the 4 replicate plates were stacked on top of each other and by looking through them it was determined whether 2 or more lids were circled in any position. For those positions all the positive values were written into an excel file.

For confirmation by dose response fresh compound in 100% DMSO was used and from an initial dilution to 2% DMSO three further dilutions in 3.16 fold steps with a 2% DMSO solution in S-buffer were prepared. In that way 4 concentrations, 20 µM, 6.3 µM, 2 µM and 0.63 µM were tested, all in 0.2% DMSO background. Both columns 1 and 12 contained 0.2% DMSO as control. Each plate

contained 20 different compounds, with 4 replica-plates of them.

Table 3

		compl	comp2	comp3	Comp4	comp5	comp6	comp7	comp8	comp9	compl	
	1	2	3	4	5	6	7	8	9	10	11	12
A	cntrl	20µM	20µM	20µM	20µM	20µM	20µM	20µM	20µM	20µM	20µM	cntrl
B	cntrl	6µM	6µM	6µM	6µM	6µM	6µM	6µM	6µM	6µM	6µM	cntrl
C	cntrl	2µM	2µM	2µM	2µM	2µM	2µM	2µM	2µM	2µM	2µM	cntrl
D	cntrl	0.6µM	0.6µM	0.6µM	0.6µM	0.6µM	0.6µM	0.6µM	0.6µM	0.6µM	0.6µM	cntrl
E	cntrl	20µM	20µM	20µM	20µM	20µM	20µM	20µM	20µM	20µM	20µM	cntrl
F	cntrl	6µM	6µM	6µM	6µM	6µM	6µM	6µM	6µM	6µM	6µM	cntrl
G	cntrl	2µM	2µM	2µM	2µM	2µM	2µM	2µM	2µM	2µM	2µM	cntrl
H	cntrl	0.6µM	0.6µM	0.6µM	0.6µM	0.6µM	0.6µM	0.6µM	0.6µM	0.6µM	0.6µM	cntrl
		compl 1	compl 2	compl 3	Compl 4	compl 5	compl 6	compl 7	compl 8	compl 9	comp2 0	

"Cntrl"-abbreviation for control

For some compounds an additional dose response with 7 concentrations was made, mostly with 2 fold dilutions to obtain 20 µM, 10 µM, 5 µM, 2.5 µM, 1.25 µM, 0.63 µM and 0.31 µM. In that case also row H contained controls. Each plate contained 10 different compounds, with 4 replica-plates of them. An example of the 26

negative controls of 16 plates showes the variability of the mean while the standard deviation remained fairly constant (Figure 5b). Furthermore, the negative controls expressed as percentage of the plate mean were approximately normal distributed (Figure 7). Therefore all data were normalized according to the calculation below, which centers value of no effect at 0 and calibrates the y-axis to standard deviations. The concentrations are on the x-axis in logarithmic scale. All 4 replicates are plotted, in addition a smoothed line through the averages is plotted.

value in SD = (number of adults of the well -1)/SD of the controls of the set
average controls of the plate

A compound was determined as confirmed and designated a hit when either the average or two of the 4 values reached 2.5 SD (corresponds to 99.3% confidence) at any concentration and a reasonable dose-response is apparent.

Results

From 23.040 compounds a total of 300 positives were obtained during the screening, of which 173 could be reconfirmed.

Table 4

library name	size	Positives	confirmed hits	% re-confirmed	hit rate
Library 1	2000	33	3	9%	0.15%
Library 2	5040	92	62	67%	1.23%
Library 3	16000	175	108	62%	0.68%
TOTAL	23040	300	173	57%	0.75%

To estimate the potency of the screen, that is to estimate what fraction of compounds that could have been identified with the assay have actually been identified during the screen, an analysis on 47 compounds defining 11 chemical clusters has been performed: 36 of these compounds have been confirmed. Another 40 compounds, which were not found to be active in the original screen but are members of those clusters, were submitted to dose response confirmation. 4 more hits have been identified. In total 40 compounds could be confirmed, 36 of the screen positives and 4 from the extra set. Hence 90% of the final hits of these clusters were detected in the original screen and 10% were missed.

Table 5

Cluster	positives	confirmed hits	similar negatives	extra hits	final hits
1	5	4	1	0	4
3	6	6	7	1	7
4	7	6	1	0	6
5	4	4	1	0	4
6	3	3	5	1	4
7	5	3	1	0	3
8	3	1	7	1	2
9	5	4	13	0	4
12	5	2	1	0	2
13	2	2	2	0	2
15	2	1	1	1	2
Total	47	36	40	4	40

Conclusions

1. A mutation in the *C. elegans* insulin receptor, *daf-2(m41)*, was used successfully in an pharmacological assay for compounds acting in the downstream pathway.
2. The assay is sensitive enough to screen at 20 µM compound concentrations, at which there were

nearly no problems due to lethality (27 of 23,040).

3. A hit rate of 0.75% from combinatorial chemistry libraries has been obtained, strongly dependent on the library.
4. The screen is specific for the insulin receptor pathway and is unlikely to yield many hits upstream e.g. stimulating insulin release.
5. The active compounds are candidates to cure insulin resistance and therefore of potential therapeutic use in type II diabetes and obesity.
6. Since the compounds bypass the need of insulin they are also of potential use in type I diabetes.
7. The major mode of compound entry in *C. elegans* is the gut which pre-selects for orally active compounds.
8. The activity is retrieved from a whole-organism readout leaving intact tissue-specific insulin signalling and feedback loops.

Table 6: Retest of 94 compounds at 20μM on 3 different daf-2 alleles, m41 at 211C, e1368 and e1370 at 251C. Values: 3: all replicates above 99% threshold, 2: median above 99.9% threshold, 1: median above 99% threshold, 0: median below 99% threshold.

ID	MW	Plate	Row	Col	*m41*	*e1368*	*e1370*
217485	547.18	1	A	2	1	1	0
211706	472.55	1	A	3	3	3	0
181141	459.51	1	A	4	3	1	0
259910	384.53	1	A	5	0	0	0
194326	393.49	1	A	6	2	0	0
217336	420.04	1	A	7	3	3	0
267546	372.51	1	A	8	0	0	0
228433	405.56	1	A	9	0	0	0
264792	436.94	1	A	10	3	0	0
255126	431.50	1	A	11	3	0	0
100718	399.88	1	B	2	3	0	0
182576	486.39	1	B	3	0	0	0
232839	475.30	1	B	4	3	1	0
217339	394.00	1	B	5	3	1	0
217341	394.00	1	B	6	3	2	0
118776	437.52	1	B	7	2	0	0
118783	452.35	1	B	8	3	2	0
118789	442.35	1	B	9	2	1	0
248144	440.89	1	B	10	3	0	0
234291	462.76	1	B	11	0	0	0
212465	367.39	1	C	2	0	0	0
144331	363.98	1	C	3	0	0	0
138263	372.51	1	C	4	2	1	0
264982	352.48	1	C	5	1	1	0
267659	386.93	1	C	6	1	0	0
115771	391.50	1	C	7	3	0	0
105359	326.40	1	C	8	3	0	0
267467	419.37	1	C	9	0	0	0
236867	480.25	1	C	10	0	0	0
225671	365.44	1	C	11	0	0	0
225858	444.33	1	D	2	0	1	0
225615	523.23	1	D	3	0	1	0
101025	431.42	1	D	4	1	0	0
255192	420.38	1	D	5	3	1	0
217850	391.27	1	D	6	3	0	0
214475	329.36	1	D	7	3	1	0
114446	479.71	1	D	8	2	0	0
261736	378.40	1	D	9	2	0	0
210145	373.84	1	D	10	0	0	0
114816	304.40	1	D	11	2	0	0

210877	445.34	1	E	2	0	0	0
189119	379.38	1	E	3	3	1	0
203845	379.38	1	E	4	1	0	0
190303	303.36	1	E	5	0	0	0
253121	524.23	1	E	6	3	1	0
228525	462.45	1	E	7	2	1	0
118761	381.89	1	E	8	2	0	0
228489	428.55	1	E	9	1	0	0
250480	332.36	1	E	10	2	1	0
118765	416.33	1	E	11	3	0	0
254230	425.24	1	F	2	0	0	0
255339	427.69	1	F	3	2	1	0
250001	383.24	1	F	4	2	0	0
255335	383.24	1	F	5	2	2	0
263986	330.86	1	F	6	0	0	0
236861	486.21	1	F	7	0	0	0
104926	280.35	1	F	8	0	1	0
133891	272.30	1	F	9	0	0	0
154290	364.27	1	F	10	2	0	0
189005	363.76	1	F	11	1	0	0
195094	346.29	1	G	2	2	0	0
203897	408.21	1	G	3	3	0	0
210775	510.21	1	G	4	1	0	0
214387	376.64	1	G	5	3	0	0
219414	318.33	1	G	6	1	0	0
228301	311.36	1	G	7	0	0	0
228488	414.53	1	G	8	1	0	0
230672	376.21	1	G	9	0	0	0
231561	365.88	1	G	10	0	0	0
236341	386.41	1	G	11	0	0	0
249726	422.19	1	H	2	1	0	0
249746	373.33	1	H	3	2	0	0
253051	311.57	1	H	4	0	0	0
257516	380.73	1	H	5	0	0	0
258687	305.36	1	H	6	0	0	0
260067	357.18	1	H	7	0	0	0
265080	346.29	1	H	8	0	1	0
268434	372.42	1	H	9	0	0	0
273546	443.05	1	H	10	0	0	0
276545	337.70	1	H	11	1	0	0
278617	430.05	2	A	2	0	0	0
279528	316.34	2	A	3	0	0	0
281078	344.25	2	A	4	3	0	0
283400	390.31	2	A	5	0	0	0
284204	301.26	2	A	6	0	0	0
284316	385.22	2	A	7	0	0	0
286676	354.15	2	A	8	0	0	0
301158	475.86	2	A	9	3	2	0
304896	432.26	2	A	10	0	0	0
307069	362.82	2	A	11	0	0	0
309471	453.32	2	B	2	0	0	0
310513	318.13	2	B	3	2	1	0
313944	416.29	2	B	4	0	0	0

316982	516.85	2	B	5	2	0	0
number or compounds active					53	25	0
percentage of compounds active					56%	27%	0%

Example 2: automatic data aquisition with Nile Red staining

Material:

Hardware:

- microtiterplates:96 well black U-shaped plates (DYNEX Microfluor7 2)
- Wallac 1420 plate reader (Victor 2):

  Nile Red protocol: excitation = 530 nm

  emission = 590 nm

  Counting time: 1 second

  CW lamp energy: 30445

  Emission aperture: damp

  Counter position: top

  Measurement height: 3 mm from bottom of the plate

Consumables:

- Nile Red (Sigma, N-3013)
- Ivermectin (ICN, 196009)

Method:

- Prepare a 100 mM solution of Nile Red (Nile Blue A Oxazone) in pure methanol. Centrifugate to remove the saturated solution from the undissolved Nile Red.
- Dilute in steps of 10 with buffer to 500 µM.
- Add 1:1 Nile Red to the worms and incubate for 30 min at room temperature.
- Add 10 µM ivermectin final concentration and incubate for 30 min at room temperature.
- Measure.

Example 3: automatic data aquisition with a vit-2::luciferase reporter

Material:

Hardware:

- microtiterplates:96 well white U-shaped plates (DYNEX Microfluor â 2)
- Wallac 1420 plate reader (Victor 2):
  Luciferase protocol
  Emission Filter: no filter
  Counting time: 3 seconds
  Emission aperture: normal

Consumables:

- Triton X-100 (BDH, 306324N)
- Dual-Luciferaseâ Reporter Assay System (Promega, E4550)

Method:

- Add Triton X-100 (1% final concentration) to lyse the worms.
- Shake for 1 minute and freeze.
- Thaw the plates and add 1:1 luciferine.
- Shake for 1 minute and measure.

Example 4: construction of ctl-1::luciferase and sod-3::luciferase reporters

1) Construction of pGQ1

1.1 PCR

PCR (turbo pfu) on N2 genomic DNA with:
oGQ1:ctl-1::GFP fw (PstI):
5' AAAACTGCAGCCAATGCATTGGAAGAGATATTTTGCGCGTCAAATATGTTTTGTGTCC3'
oGQ2bis:ctl-1::GFP rv (BamHI)
5'CGCGGATCCGGCCGATTCTCCAGCGACCG3'

1.2 Cloning
- Digest of the PCR fragment with PstI and BamHI
- Ligation into pDW2020 and transformation into DH10B

2) Construction of pGQ2

2.1 PCR

PCR (turbo pfu) on N2 genomic DNA with:
oGQ3:ctl-1::luciferase fw (StuI):
5' CCAGGCCTGAGATATTTTGCGCGTCAAATATGTTTTGTGTCC3'
oGQ4:ctl-1::luciferase rv (SacI)

5'CGGAGCTCCGATTGGATGTGGTGAGCAGG3'

2.2 Cloning

- Digest of the PCR fragment with StuI and SacI
- Ligation into pCluc6 and transformation into DH10B

3) Construction of pGQ3

3.1 PCR

PCR (turbo pfu) on N2 genomic DNA with:

oGQ7:sod-3 fw:

5'GCAGAATTTGCAAAACGAGCAGGAAAGTC3'

oGQ6:sod-3::luciferase rv (AscI)

5'TTGGCGCGCCAAGCCTTAATAGTGTCCATCAGC3'

3.2 Cloning

- Digest of the PCR fragment with PstI and AscI
- Ligation into pDW2020 and transformation into HD10B

4) Construction of pGQ4

4.1 PCR

PCR (turbo pfu) on N2 genomic DNA with:

oGQ7:sod-3 fw:

5'GCAGAATTTGCAAAACGAGCAGGAAAGTC3'

oGQ8:sod-3::luciferase rv (SacI)

5'CTGAGCTCGGCTTAATAGTGTCCATCAGC3'

4.2 Cloning

- Digest of the PCR fragment with PstI and SacII

- Ligation into pCluc6 and transformation into HD10B

Example 5: Construction of pCluc6

Vector:

- Restriction digest of pCluc2 with HindIII
- Purification, protocol: Jetsorb

Insert:

- PCR the vit-2 promoter (248 bp in front of exon1 just before ATG) with primers (designed from ACeDB C42D8.2) that contain HindIII RE sites out of N2 genomic DNA:

vit-2F: 5'CCCCCAAGCTTCCATGTGCTAGCTGAGTTTCATCATGTCC3'

vit-2R: 5'CCCCCCAAGCTTGGCTGAACCGTGATTGG3'

- Restriction digest on PCR product with HindIII
- Purification, protocol: Jetsorb

pCluc6:

- T4 DNA ligation of vector and insert
- Transformation into DH10B
- Mini DNA preparation, protocol: Wizard SV Miniprep
- determine direction of insert by RE cleavage XbaI/NheI
- Maxi DNA preparation, protocol: Jetstar
- Check maxiprep by sequencing with o-PUCI primer.

Standard methods and worm strains

Standard methods for culturing nematodes are described in Methods in Cell biology Vol. 48, 1995, ed. by Epstein and Shakes, Academic press. Standard methods are known for creating mutant worms with mutations in

selected *C. elegans* genes, for example see J. Sutton and J. Hodgkin in "The Nematode *Caenorhabditis elegans*", Ed. by William B. Wood and the Community of *C. elegans* Researchers CSHL, 1988 594-595; Zwaal et al, "Target - Selected Gene Inactivation in *Caenorhabditis elegans* by using a Frozen Transposon Insertion Mutant Bank" 1993, Proc. Natl. Acad. Sci. USA <u>90</u> pp 7431 -7435; Fire et al, Potent and Specific Genetic Interference by Double-Stranded RNA in *C. elegans* 1998, Nature <u>391</u>, 860-811. A population of worms can be subjected to random mutagenesis by using EMS, TMP-UV or radiation (Methods in Cell Biology, Vol 48, ibid). Several selection rounds of PCR could then be performed to select a mutant worm with a deletion in a desired gene.

A range of specific *C. elegans* mutants are available from the *C. elegans* mutant collection at the *C. elegans* Genetic Center, University of Minnesota, St Paul, Minnesota.

E. coli strain OP50 can be obtained from the *C. elegans* Genetics Center, University of Minnesota, St Paul, Minnesota, USA.

CLAIMS:

1. A method for the identification of a compound which is capable of modulating insulin signalling pathways, which method comprises: providing *C. elegans* dauer larvae; contacting said larvae with a test compound; and screening for release from the dauer larval state, wherein the *C. elegans* dauer larvae possess a sensitized genetic background, as compared to the reference *daf-2* mutant *e1370*.

2. Method according to claim 1, in which the dauer larvae belong to a nematode strain which has an Insulin Sensitivity Value ("ISV") that is greater than the ISV for the reference nematode strain CB1370, in particular more than 1% greater, preferably more than 5% greater, more preferably more than 10% greater, even more preferably more than 20% greater.

3. Method according to claim 1 and/or 2, in which the dauer larvae belong to a nematode strain which has an ISV that is >30 %, preferably >40%, even more preferably >50%.

4. A method as claimed in claim 1 wherein the *C.elegans* dauer larvae are daf-2(m41) mutants.

5. A method as claimed in claim 1 wherein the *C. elegans* dauer larvae comprise a daf-2 class I allele other than daf-2(m41).

6. A method as claimed in claim 1 wherein the *C. elegans* dauer larvae comprise at least one loss-of-function or reduction-of-function mutation in a gene(s) downstream of the insulin receptor in the insulin signalling pathway.

7. A method as claimed in claim 6 wherein the *C. elegans* dauer larvae comprise a loss-of-function or reduction-of-function mutation in the *age-1* gene.

8. A method as claimed in claim 6 wherein the *C.elegans* dauer larvae comprise loss-of-function or reduction-of-function mutations in the *akt-1* gene and the *akt-2* gene.

9. A method as claimed in claim 6 wherein the *C. elegans* dauer larvae comprise a loss-of-function or reduction-of-function mutation in the *pdk-1* gene.

10. A method as claimed in claim 9 wherein the *C. elegans* dauer larvae are *pdk-1(sa680)* mutants.

11. A method as claimed in claim 1 wherein the *C. elegans* dauer larvae are larvae wherein the dauer phenotype is induced by treatment with an inhibitor inhibitor of at least one component of the insulin receptor signalling pathway.

12. A method as claimed in claim 11 wherein the inhibitor compound is an inhibitor of the *C. elegans* PI3-kinase.

13. A method as claimed in claim 12 wherein the inhibitor compound is wortmannin or LY294002.

14. A method as claimed in claim 1 wherein expression of at least one gene downstream of the insulin receptor in the insulin receptor signalling pathway in said *C. elegans* dauer larvae is inhibited by RNAi inhibition.

15. A method as claimed in claim 1 wherein the *C. elegans* dauer larvae comprise a gain-of-function mutation in the *daf-16* gene.

16. A method as claimed in claim 1 wherein the *C. elegans* dauer larvae comprise a gain-of-function mutation in the *daf-18* gene.

17. A method as claimed in claim 1 wherein the *C. elegans* dauer larvae comprise a gain-of-function mutation in the *C. elegans* homologue of the SHIP2 gene.

18. A method as claimed in claim 1 wherein the *C. elegans* larvae dauer comprise a gain-of-function mutation in the *C. elegans* homologue of the PTP-1B gene.

19. A method as claimed in claim 1 wherein the *C. elegans* dauer larvae exhibit a defect in perception of environmental signals.

20. A method as claimed in claim 19 wherein the said *C. elegans* dauer larvae comprise a mutation in the *tph-1* gene.

21. A method as claimed in claim 20 wherein the said *C. elegans* dauer larvae are *tph-1(mg280)* mutants.

22. A method as claimed in claim 1 wherein the *C. elegans* dauer larvae comprise a daf-c mutation in a daf gene selected from the group consisting of *daf-1*, *daf-4*, *daf-7*, *daf-8*, *daf-11*, *daf-14*, *daf-21*, *daf-19* and *daf-28*.

23. A method as claimed in claim 1 wherein the *C. elegans* dauer larvae comprise a mutation in a gene encoding a neuronal G-protein.

24. A method as claimed in claim 1 wherein the c. elegans dauer larvae are *unc-64(e264); unc-31 (e928)* mutants.

25. A method as claimed in any one of claims 1 to 24 wherein the step of screening for release from the dauer larval state comprises screening for adult *C. elegans.*

26. A method as claimed in any one of claims 1 to 24 wherein the step of screening for release from the dauer larval state comprises screening for changes in fat storage.

27. A method as claimed in any one of claims 1 to 24 wherein said *C. elegans* dauer larvae further comprise a reporter transgene comprising a promoter which is capable of directing strong gene expression in adult *C. elegans* and no or weak expression in dauer larvae or vice versa operably linked to a reporter gene and the step of screening for release from the dauer larval state comprises screening for changes in expression of the said reporter gene.

28. A method for the identification of a compound which is capable of modulating insulin signalling pathways, which method comprises:
providing *C. elegans* dauer larvae;
contacting said larvae with a test compound; and screening for release from the dauer larval state, wherein conditions of the assay are selected such that a basal level of release from the dauer larval state is observed in the absence of the test compound.

29. A method as claimed in claim 28 wherein the basal level of release from the dauer larval state is between 0.1% and 40%.

30. A method as claimed in claim 29 wherein the basal level of release from the dauer larval state is between 1% and 30%.

31. A method as claimed in claim 30 wherein the basal level of release from the dauer larval state is between 2% and 20%.

32. A method as claimed in any one of claims 28 to 31 wherein the *C. elegans* dauer larvae are *daf-2(m41)* mutants.

33. A method as claimed in any one of claims 28 to 31 wherein the *C. elegans* dauer larvae are *daf-2; daf-18* double mutants.

34. A method as claimed in any one of claims 28 to 31 wherein the *C. elegans* dauer larvae are Daf-d mutants.

35. A method as claimed in any one of claims 28 to 31 wherein the *C. elegans* dauer larvae comprise a gain-of-function mutation in the *pdk-1* gene.

36. A method as claimed in claim 35 wherein the *C. elegans* dauer larvae are *pdk-1(mg142)* mutants.

37. A method as claimed in any one of claims 28 to 31 wherein the *C. elegans* dauer larvae comprise a gain-of-function mutation in the *akt-1* gene.

38. A method as claimed in claim 37 wherein the *C. elegans* dauer larvae are *akt-1(mg144)* mutants.

39. A method as claimed in any one of claims 28 to 31 wherein the *C. elegans* dauer larvae are *daf-16; daf-2* double mutants and further comprise a transgene capable of expressing a mammalian homolog of the daf-16 protein.

40. A method as claimed in claim 39 wherein the mammalian homolog of the daf-16 protein is the human FKHR protein, the human FKHRL1 protein or the human AFX protein.

41. A method as claimed in claim 28 wherein said *C. elegans* dauer larvae are larvae which have been treated with pheromone to reduce that fraction of worms growing to adults to below 40%.

42. A method as claimed in claim 41 wherein said *C. elegans* dauer larvae are larvae which have been treated with pheromone to reduce that fraction of worms growing to adults to below 30%.

43. A method as claimed in claim 42 wherein said *C. elegans* dauer larvae are larvae which have been treated with pheromone to reduce that fraction of worms growing to adults to below 20%.

44. A method as claimed in any one of claims 28 to 43 wherein the step of screening for release from the dauer larval state comprises screening for adult *C. elegans*.

45. A method as claimed in any one of claims 28 to 43 wherein said *C. elegans* larvae further comprise a reporter transgene comprising a promoter which is capable of directing strong gene expression in adult *C. elegans* and no or weak expression in dauer larvae or vice versa operably linked to a reporter gene and the step of screening for rescue of the *daf-2* mutation

comprises screening for expression of the said reporter gene.

46. A method as claimed in any one of claims 28 to 43 wherein the step of screening for release from the dauer larval state comprises screening for changes in fat storage.

47. A method for the identification of a compound which is capable of modulating insulin signalling pathways, which method comprises:

a) providing a sample of nematode worms (preferably eggs, L1 or L2 worms, and most preferably L1 worms);
b) keeping said sample under conditions such, without the presence of any compound(s) to be tested, at least 50%, and preferably at least 60 %, and more preferably at least 70 %, even more preferably at least 80 %, such as 85-100% of the nematodes present in said sample would enter the dauer state (at least during the time used for the assay);
c) exposing the sample to the compound(s) to be tested;
d) measuring either the number of worms that enter the dauer state, and/or measuring the number of worms that grow into adults.

48. Method according to claim 47, in which the conditions used in step b) are such that, in the presence of a reference compound at a suitable concentration, the amount of worms that enter the dauer state is at least 10% less, preferably at least

20% less, more preferably at least 30% less, than the amount of worms that would enter the dauer state without the presence of any such reference compound (at least during the time used for the assay).

49. Method according to claim 46 and/or 47, in which the conditions used in step b) are such that, in the presence of a reference compound at a suitable concentration, the amount of worms that enter the dauer state is less than 50%, preferably less than 40%, even more preferably less than 30% (at least during the time used for the assay).

50. Method according to any of claims 47-49, in which the nematode worms that form the sample belong to a nematode strain that has an Insulin Sensitivity Value ("ISV") that is greater than the ISV for the reference nematode strain CB1370, in particular more than 1% greater, preferably more than 5% greater, more preferably more than 10% greater, even more preferably more than 20% greater.

51. Method according to any of claims 47-50, in which the nematode worms that form the sample belong to a nematode strain which has an ISV that is >30 %, preferably >40%, even more preferably >50%.

52. Method according to any of claims 47-50, in which the nematodes used in the sample are daf-2(m41) mutants.

53. Use of at least one nematode worm, which has

an increased sensitivity of the insulin signalling pathway, in an assay for the identification of a compound which is capable of modulating insulin signalling pathways.

54. Use according to claim 53, in which the nematode worm belongs to a strain that has an Insulin Sensitivity Value ("ISV") that is greater than the ISV for the reference nematode strain CB1370, in particular more than 1% greater, preferably more than 5% greater, more preferably more than 10% greater, even more preferably more than 20% greater.

55. Use according to claim 53 and/or 54, in which the nematode worm belongs to a strain that has an Insulin Sensitivity Value ("ISV") that is >30 %, preferably >40%, even more preferably >50%

56. Use according to any of claims 53-55, in which the nematode worm used is a daf-2(m41) mutant.

57. Use according to any of claims 53-56, in an assay that is carried out in a multi-well plate format.

58. Use according to any of claims 53-57, in an assay that is carried out in an automated fashion.

59. Use according to any of claims 53-58, in an assay based on the dauer phenotype as a biological read out, such as on the entry into, the bypass of and/or the rescue from the dauer state, and/or on any

other property which results from and/or is associated with the so-called dauer decision.

60. Use according to claim 59, in an assay based on entry into the dauer state and/or bypass of the dauer state as a biological read out.

61. Use according to claim 59, in an assay based on rescue from the dauer state as a biological read out.

62. Use according to any of claims 53-61, for the identification of a small molecule and/or a small peptide.

Figure 1: The insulin receptor pathway

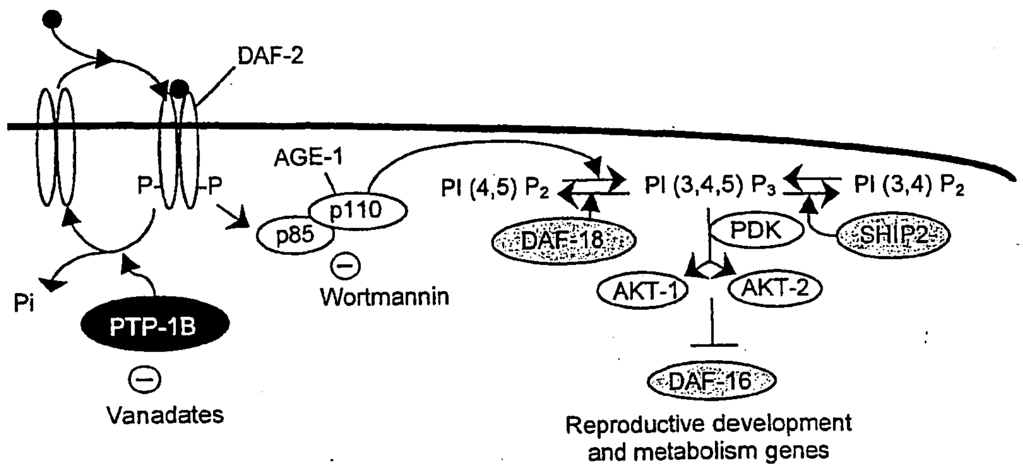


Figure 2. The reference allele of *daf-2* is *e1370*

```
Locus: daf-2
daf-2                                    Biblio  Attach...  Quit

Name  Gene_class  daf
Type  Gene  Reference_Allele  e1370
            Phenotype  e1370ts : constitutive dauer formation
                         at 25x; reversible by shift to 15x. ES3
                         (L3). MA19.
                       See also e1032, e1286, e1365, e1368,
                         e1370, e1391
                       [C.elegansIII] e1370ts : constitutive
                         dauer formation at 25C; reversible by
                         shift to 15C. Increased lifespanat 20C;
                         increased thermotolerance, UV
                         resistance. Non-Srf.  Synthetic lethal
                         with daf-12. ES3 (L3). DA>40: e1032,
                         e1286, e1365, sa230 (100%Daf-c at all
                         temperatures), sa223 (sterile), m65
                         (nonconditional), etc. Most alleles
                         (not e1370) hypersensitive to dauer
                         pheromone. [Larsen et al. 1995; Malone
                         and Thomas 1994; CF; JC]
Molecular_information  Sequence  EMBL:AF012437.1
                                 EMBL:AF012437.2
                                 Y55D5A_391.b
Map  III  Position  -9.88234  Error  0.059406
Positive  Inside_rearr  nDf11
          Positive_clone  C44B11
                          H05C05
Negative  Outside_rearr  tDf9
Mapping_data  Well_ordered
              2_point  -----> 4
              Multi_point  -----> 18
              Pos_neg_data  -----> 12
Allele  -----> 8
Strain  -----> 13
Reference  -----> 182
```

Figure 3: Na-ortho-vanadate rescues insulin resistance caused by *daf-2(m41)*

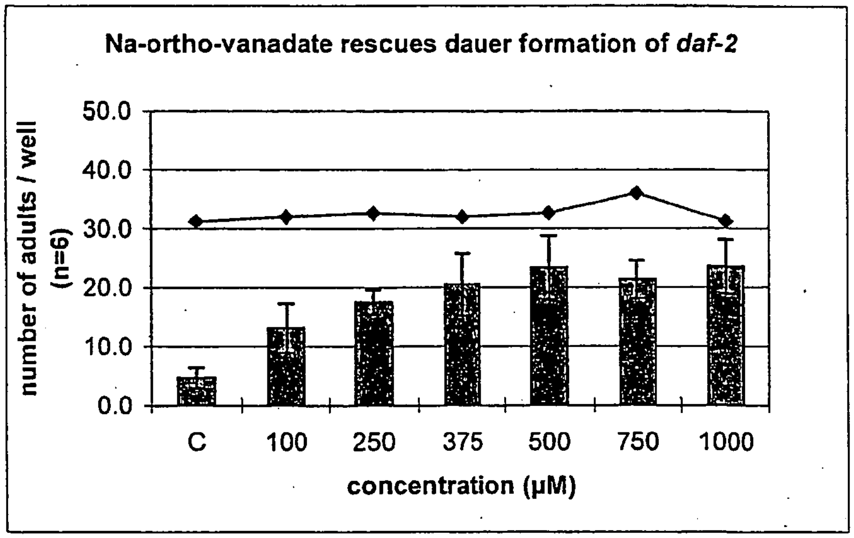


Figure 4: Wortmannin further enhances insulin resistance

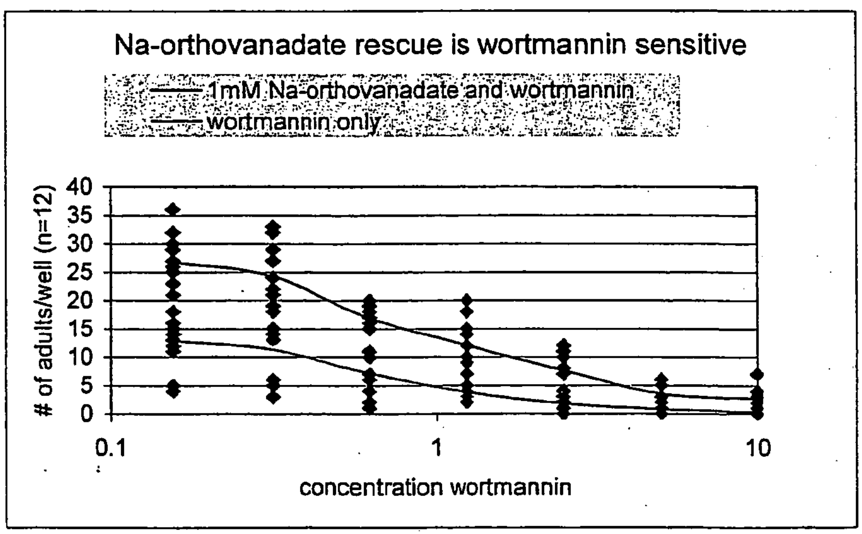


Figure 5: Scatter plots of mean and variance of controls: a (left): screening, b (right): DRC

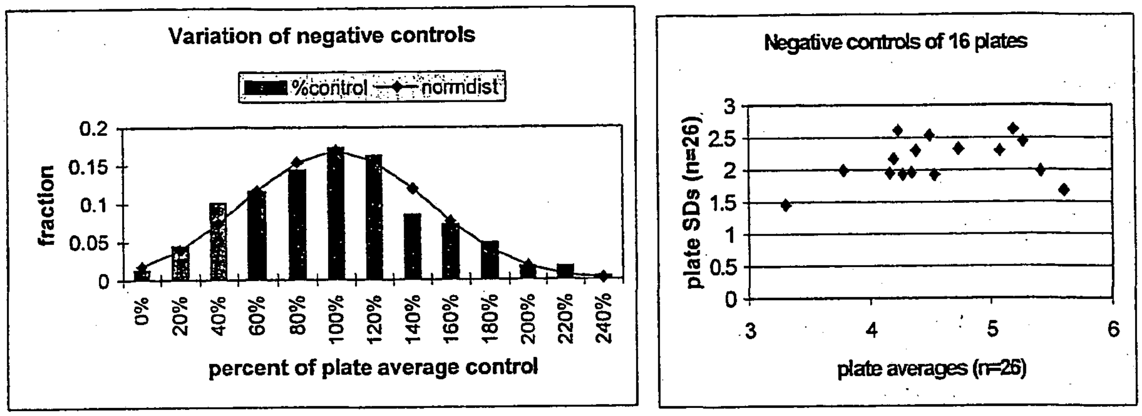


Figure 6: distribution of controls and a maximum likelyhood fit of a negative binomial distribution

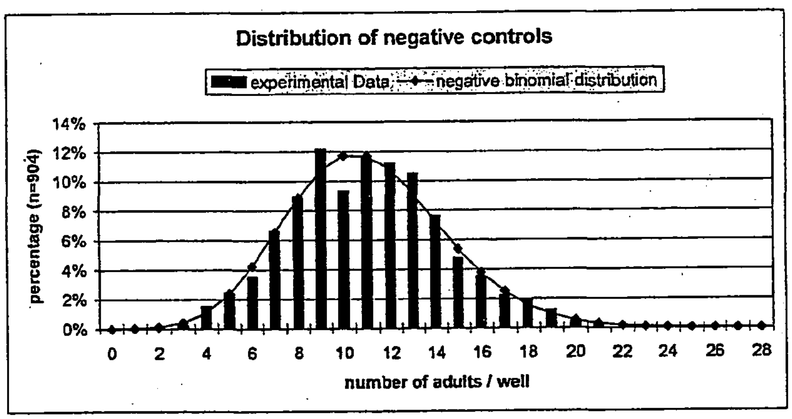


Figure 7: distribution of controls in percent of the average of the plate.

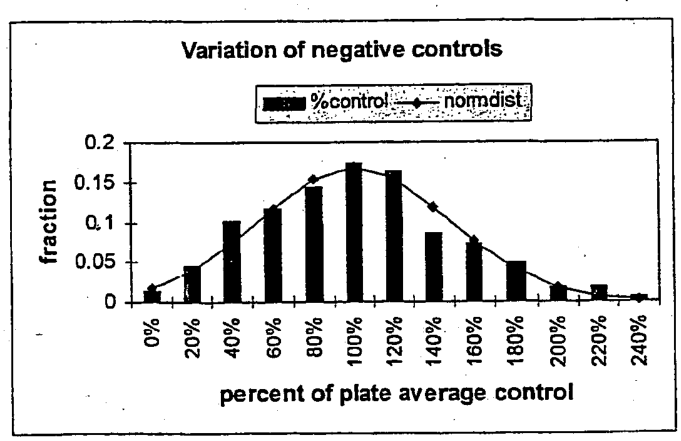


Figure 8

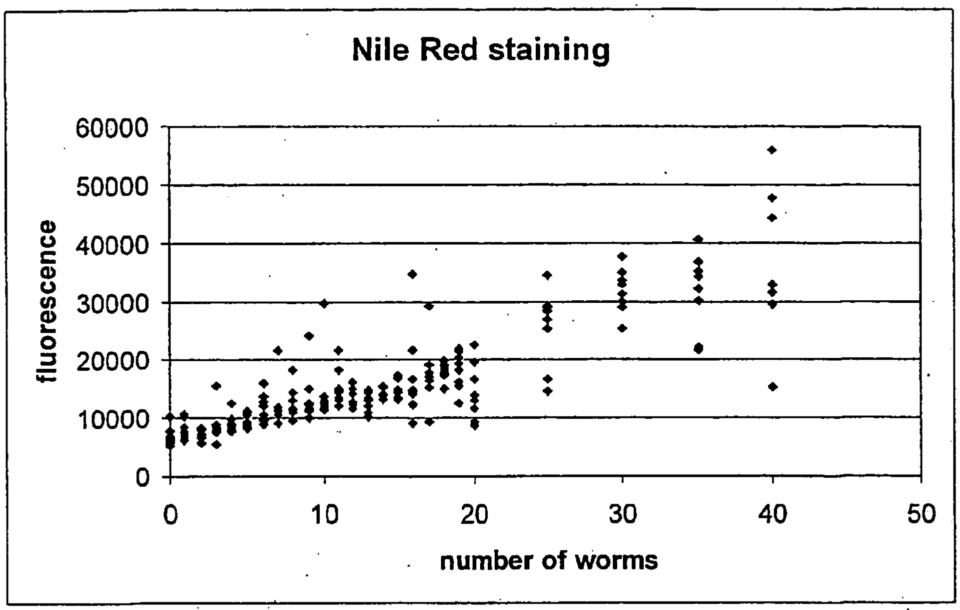

Nile Red staining
fluorescence
60000
50000
40000
30000
20000
10000
0
0
10
20
30
40
50
number of worms

Figure 9

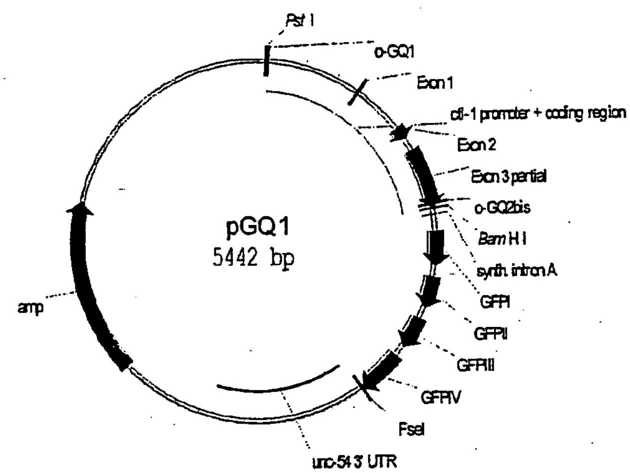


Figure 10

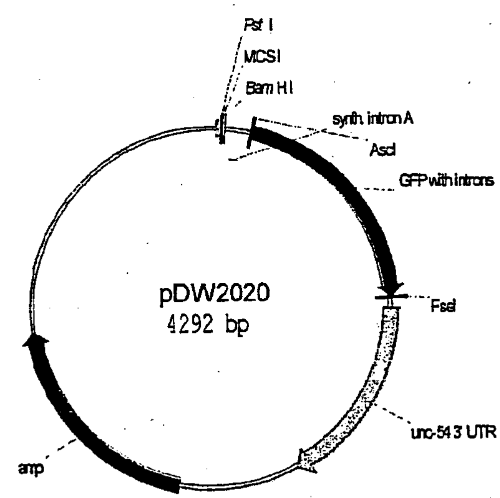


Fig. 11

pDW2020 sequence:

```
                                        MCS I
                          ====================================
                                        PstI             BamHI
                                     ~~~~~~~               ~~~
  1  ATGACCATGA TTACGCCAAG CTTGCATGCC TGCAGGTCGA CTCTAGAGGA
     TACTGGTACT AATGCGGTTC GAACGTACGG ACGTCCAGCT GAGATCTCCT

     MCS I                                  synth.  intron A
     ===                                ========================
     BamHI
     ~~~
 51  TCCCCGGGAT TGGCCAAAGG ACCCAAAGGT ATGTTTCGAA TGATACTAAC
     AGGGGCCCTA ACCGGTTTCC TGGGTTTCCA TACAAAGCTT ACTATGATTG

     synth. intron  A
     =====================
101  ATAACATAGA ACATTTTCAG GAGGACCCTT GGCTAGCGTC GACGGTACCA
     TATTGTATCT TGTAAAAGTC CTCCTGGGAA CCGATCGCAG CTGCCATGGT

          AscI                               GFP with  introns
          =============================================
151  TGGGGCGCGC CATGAGTAAA GGAGAAGAAC TTTTCACTGG AGTTGTCCCA
     ACCCCGCGCG GTACTCATTT CCTCTTCTTG AAAAGTGACC TCAACAGGGT

                                             GFP with  introns
     ======================================================
201  ATTCTTGTTG AATTAGATGG TGATGTTAAT GGGCACAAAT TTTCTGTCAG
     TAAGAACAAC TTAATCTACC ACTACAATTA CCCGTGTTTA AAAGACAGTC

                                             GFP with  introns
     ======================================================
251  TGGAGAGGGT GAAGGTGATG CAACATACGG AAAACTTACC CTTAAATTTA
     ACCTCTCCCA CTTCCACTAC GTTGTATGCC TTTTGAATGG GAATTTAAAT

                                             GFP with  introns
     ======================================================
301  TTTGCACTAC TGGAAAACTA CCTGTTCCAT GGGTAAGTTT AAACATATAT
     AAACGTGATG ACCTTTTGAT GGACAAGGTA CCCATTCAAA TTTGTATATA

                                             GFP with  introns
     ======================================================
351  ATACTAACTA ACCCTGATTA TTTAAATTTT CAGCCAACAC TTGTCACTAC
     TATGATTGAT TGGGACTAAT AAATTTAAAA GTCGGTTGTG AACAGTGATG

                                             GFP with  introns
     ======================================================
401  TTTCTGTTAT GGTGTTCAAT GCTTCTCGAG ATACCCAGAT CATATGAAAC
     AAAGACAATA CCACAAGTTA CGAAGAGCTC TATGGGTCTA GTATACTTTG

                                             GFP with  introns
     ======================================================
```

fig. 11 continued

```
451  GGCATGACTT TTTCAAGAGT GCCATGCCCG AAGGTTATGT ACAGGAAAGA
     CCGTACTGAA AAAGTTCTCA CGGTACGGGC TTCCAATACA TGTCCTTTCT

                                          GFP with  introns
     ==================================================
501  ACTATATTTT TCAAAGATGA CGGGAACTAC AAGACACGTA AGTTTAAACA
     TGATATAAAA AGTTTCTACT GCCCTTGATG TTCTGTGCAT TCAAATTTGT

                                          GFP with  introns
     ==================================================
551  GTTCGGTACT AACTAACCAT ACATATTTAA ATTTTCAGGT GCTGAAGTCA
     CAAGCCATGA TTGATTGGTA TGTATAAATT TAAAAGTCCA CGACTTCAGT

     GFP with introns
     ==================================================
601  AGTTTGAAGG TGATACCCTT GTTAATAGAA TCGAGTTAAA AGGTATTGAT
     TCAAACTTCC ACTATGGGAA CAATTATCTT AGCTCAATTT TCCATAACTA

     GFP with introns
     ==================================================
651  TTTAAAGAAG ATGGAAACAT TCTTGGACAC AAATTGGAAT ACAACTATAA
     AAATTTCTTC TACCTTTGTA AGAACCTGTG TTTAACCTTA TGTTGATATT

     GFP with introns
     ==================================================
701  CTCACACAAT GTATACATCA TGGCAGACAA ACAAAAGAAT GGAATCAAAG
     GAGTGTGTTA CATATGTAGT ACCGTCTGTT TGTTTTCTTA CCTTAGTTTC

     GFP with introns
     ==================================================
751  TTGTAAGTTT AAACTTGGAC TTACTAACTA ACGGATTATA TTTAAATTTT
     AACATTCAAA TTTGAACCTG AATGATTGAT TGCCTAATAT AAATTTAAAA

     GFP with introns
     ==================================================
801  CAGAACTTCA AAATTAGACA CAACATTGAA GATGGAAGCG TTCAACTAGC
     GTCTTGAAGT TTTAATCTGT GTTGTAACTT CTACCTTCGC AAGTTGATCG

     GFP with introns
     ==================================================
851  AGACCATTAT CAACAAAATA CTCCAATTGG CGATGGCCCT GTCCTTTTAC
     TCTGGTAATA GTTGTTTTAT GAGGTTAACC GCTACCGGGA CAGGAAAATG

     GFP with introns
     ==================================================
901  CAGACAACCA TTACCTGTCC ACACAATCTG CCCTTTCGAA AGATCCCAAC
     GTCTGTTGGT AATGGACAGG TGTGTTAGAC GGGAAAGCTT TCTAGGGTTG

     GFP with introns
     ==================================================
951  GAAAAGAGAG ACCACATGGT CCTTCTTGAG TTTGTAACAG CTGCTGGGAT
     CTTTTCTCTC TGGTGTACCA GGAAGAACTC AAACATTGTC GACGACCCTA

     GFP with introns                     FseI
```

Fig. 11 Continued

```
     ==========================================
1001 TACACATGGC ATGGATGAAC TATACAAATA GGGCCGGCCG AGCTCCGCAT
     ATGTGTACCG TACCTACTTG ATATGTTTAT CCCGGCCGGC TCGAGGCGTA

                                           unc-54  3' UTR
                                 ==============================
1051 CGGCCGCTGT CATCAGATCG CCATCTCGCG CCCGTGCCTC TGACTTCTAA
     GCCGGCGACA GTAGTCTAGC GGTAGAGCGC GGGCACGGAG ACTGAAGATT

                                           unc-54  3' UTR
     ======================================================
1101 GTCCAATTAC TCTTCAACAT CCCTACATGC TCTTTCTCCC TGTGCTCCCA
     CAGGTTAATG AGAAGTTGTA GGGATGTACG AGAAAGAGGG ACACGAGGGT

                                           unc-54  3' UTR
     ======================================================
1151 CCCCCTATTT TTGTTATTAT CAAAAAAACT TCTTCTTAAT TTCTTTGTTT
     GGGGGATAAA AACAATAATA GTTTTTTTGA AGAAGAATTA AAGAAACAAA

                                           unc-54  3' UTR
     ======================================================
1201 TTTAGCTTCT TTTAAGTCAC CTCTAACAAT GAAATTGTGT AGATTCAAAA
     AAATCGAAGA AAATTCAGTG GAGATTGTTA CTTTAACACA TCTAAGTTTT

                                           unc-54  3' UTR
     ======================================================
1251 ATAGAATTAA TTCGTAATAA AAAGTCGAAA AAAATTGTGC TCCCTCCCCC
     TATCTTAATT AAGCATTATT TTTCAGCTTT TTTTAACACG AGGGAGGGGG

                                           unc-54  3' UTR
     ======================================================
1301 CATTAATAAT AATTCTATCC CAAAATCTAC ACAATGTTCT GTGTACACTT
     GTAATTATTA TTAAGATAGG GTTTTAGATG TGTTACAAGA CACATGTGAA

                                           unc-54  3' UTR
     ======================================================
1351 CTTATGTTTT TTTTACTTCT GATAAATTTT TTTTGAAACA TCATAGAAAA
     GAATACAAAA AAAATGAAGA CTATTTAAAA AAAACTTTGT AGTATCTTTT

                                      unc-54 3'  UTR
     ======================================================
1401 AACCGCACAC AAAATACCTT ATCATATGTT ACGTTTCAGT TTATGACCGC
     TTGGCGTGTG TTTTATGGAA TAGTATACAA TGCAAAGTCA AATACTGGCG

     unc-54 3'  UTR
     ======================================================
1451 AATTTTTATT TCTTCGCACG TCTGGGCCTC TCATGACGTC AAATCATGCT
     TTAAAAATAA AGAAGCGTGC AGACCCGGAG AGTACTGCAG TTTAGTACGA

     unc-54 3'  UTR
     ======================================================
1501 CATCGTGAAA AAGTTTTGGA GTATTTTTGG AATTTTTCAA TCAAGTGAAA
     GTAGCACTTT TTCAAAACCT CATAAAAACC TTAAAAAGTT AGTTCACTTT
```

Fig. 11 continued

```
     unc-54 3'  UTR
     ==================================================
1551 GTTTATGAAA TTAATTTTCC TGCTTTTGCT TTTTGGGGGT TTCCCCTATT
     CAAATACTTT AATTAAAAGG ACGAAAACGA AAAACCCCCA AAGGGGATAA

     unc-54 3'  UTR
     ==================================================
1601 GTTTGTCAAG AGTTTCGAGG ACGGCGTTTT TCTTGCTAAA ATCACAAGTA
     CAAACAGTTC TCAAAGCTCC TGCCGCAAAA AGAACGATTT TAGTGTTCAT

     unc-54 3'  UTR
     ==================================================
1651 TTGATGAGCA CGATGCAAGA AAGATCGGAA GAAGGTTTGG GTTTGAGGCT
     AACTACTCGT GCTACGTTCT TTCTAGCCTT CTTCCAAACC CAAACTCCGA

     unc-54 3'  UTR
     ==================================================
1701 CAGTGGAAGG TGAGTAGAAG TTGATAATTT GAAAGTGGAG TAGTGTCTAT
     GTCACCTTCC ACTCATCTTC AACTATTAAA CTTTCACCTC ATCACAGATA

     unc-54 3'  UTR
     ==================================================
1751 GGGGTTTTTG CCTTAAATGA CAGAATACAT TCCCAATATA CCAAACATAA
     CCCCAAAAAC GGAATTTACT GTCTTATGTA AGGGTTATAT GGTTTGTATT

     unc-54 3'  UTR
     ======
1801 CTGTTTCCTA CTAGTCGGCC GTACGGGCCC TTTCGTCTCG CGCGTTTCGG
     GACAAAGGAT GATCAGCCGG CATGCCCGGG AAAGCAGAGC GCGCAAAGCC

1851 TGATGACGGT GAAAACCTCT GACACATGCA GCTCCCGGAG ACGGTCACAG
     ACTACTGCCA CTTTTGGAGA CTGTGTACGT CGAGGGCCTC TGCCAGTGTC

1901 CTTGTCTGTA AGCGGATGCC GGGAGCAGAC AAGCCCGTCA GGGCGCGTCA
     GAACAGACAT TCGCCTACGG CCCTCGTCTG TTCGGGCAGT CCCGCGCAGT

1951 GCGGGTGTTG GCGGGTGTCG GGGCTGGCTT AACTATGCGG CATCAGAGCA
     CGCCCACAAC CGCCCACAGC CCCGACCGAA TTGATACGCC GTAGTCTCGT

2001 GATTGTACTG AGAGTGCACC ATATGCGGTG TGAAATACCG CACAGATGCG
     CTAACATGAC TCTCACGTGG TATACGCCAC ACTTTATGGC GTGTCTACGC

2051 TAAGGAGAAA ATACCGCATC AGGCGGCCTT AAGGGCCTCG TGATACGCCT
     ATTCCTCTTT TATGGCGTAG TCCGCCGGAA TTCCCGGAGC ACTATGCGGA

2101 ATTTTTATAG GTTAATGTCA TGATAATAAT GGTTTCTTAG ACGTCAGGTG
     TAAAAATATC CAATTACAGT ACTATTATTA CCAAAGAATC TGCAGTCCAC

2151 GCACTTTTCG GGGAAATGTG CGCGGAACCC CTATTTGTTT ATTTTTCTAA
     CGTGAAAAGC CCCTTTACAC GCGCCTTGGG GATAAACAAA TAAAAAGATT

2201 ATACATTCAA ATATGTATCC GCTCATGAGA CAATAACCCT GATAAATGCT
     TATGTAAGTT TATACATAGG CGAGTACTCT GTTATTGGGA CTATTTACGA
```

Fig. 11 continued

```
                                                      amp
                                     ========================
2251  TCAATAATAT TGAAAAAGGA AGAGTATGAG TATTCAACAT TTCCGTGTCG
      AGTTATTATA ACTTTTTCCT TCTCATACTC ATAAGTTGTA AAGGCACAGC

                                                      amp
      ======================================================
2301  CCCTTATTCC CTTTTTTGCG GCATTTTGCC TTCCTGTTTT TGCTCACCCA
      GGGAATAAGG GAAAAAACGC CGTAAAACGG AAGGACAAAA ACGAGTGGGT

                                                      amp
      ======================================================
2351  GAAACGCTGG TGAAAGTAAA AGATGCTGAA GATCAGTTGG GTGCACGAGT
      CTTTGCGACC ACTTTCATTT TCTACGACTT CTAGTCAACC CACGTGCTCA

                                                      amp
      ======================================================
2401  GGGTTACATC GAACTGGATC TCAACAGCGG TAAGATCCTT GAGAGTTTTC
      CCCAATGTAG CTTGACCTAG AGTTGTCGCC ATTCTAGGAA CTCTCAAAAG

                                                      amp
      ======================================================
2451  GCCCCGAAGA ACGTTTTCCA ATGATGAGCA CTTTTAAAGT TCTGCTATGT
      CGGGGCTTCT TGCAAAAGGT TACTACTCGT GAAAATTTCA AGACGATACA

                                                      amp
      ======================================================
2501  GGCGCGGTAT TATCCCGTAT TGACGCCGGG CAAGAGCAAC TCGGTCGCCG
      CCGCGCCATA ATAGGGCATA ACTGCGGCCC GTTCTCGTTG AGCCAGCGGC

                                                      amp
      ======================================================
2551  CATACACTAT TCTCAGAATG ACTTGGTTGA GTACTCACCA GTCACAGAAA
      GTATGTGATA AGAGTCTTAC TGAACCAACT CATGAGTGGT CAGTGTCTTT

                                                      amp
      ======================================================
2601  AGCATCTTAC GGATGGCATG ACAGTAAGAG AATTATGCAG TGCTGCCATA
      TCGTAGAATG CCTACCGTAC TGTCATTCTC TTAATACGTC ACGACGGTAT

                                                      amp
      ======================================================
2651  ACCATGAGTG ATAACACTGC GGCCAACTTA CTTCTGACAA CGATCGGAGG
      TGGTACTCAC TATTGTGACG CCGGTTGAAT GAAGACTGTT GCTAGCCTCC

        amp
      ======================================================
2701  ACCGAAGGAG CTAACCGCTT TTTTGCACAA CATGGGGGAT CATGTAACTC
      TGGCTTCCTC GATTGGCGAA AAAACGTGTT GTACCCCCTA GTACATTGAG

      amp
      ======================================================
2751  GCCTTGATCG TTGGGAACCG GAGCTGAATG AAGCCATACC AAACGACGAG
      CGGAACTAGC AACCCTTGGC CTCGACTTAC TTCGGTATGG TTTGCTGCTC
```

Fig. 11. continued

```
     amp
     ==================================================
2801 CGTGACACCA CGATGCCTGT AGCAATGGCA ACAACGTTGC GCAAACTATT
     GCACTGTGGT GCTACGGACA TCGTTACCGT TGTTGCAACG CGTTTGATAA

     amp
     ==================================================
2851 AACTGGCGAA CTACTTACTC TAGCTTCCCG GCAACAATTA ATAGACTGGA
     TTGACCGCTT GATGAATGAG ATCGAAGGGC CGTTGTTAAT TATCTGACCT

     amp
     ==================================================
2901 TGGAGGCGGA TAAAGTTGCA GGACCACTTC TGCGCTCGGC CCTTCCGGCT
     ACCTCCGCCT ATTTCAACGT CCTGGTGAAG ACGCGAGCCG GGAAGGCCGA

     amp
     ==================================================
2951 GGCTGGTTTA TTGCTGATAA ATCTGGAGCC GGTGAGCGTG GGTCTCGCGG
     CCGACCAAAT AACGACTATT TAGACCTCGG CCACTCGCAC CCAGAGCGCC

     amp
     ==================================================
3001 TATCATTGCA GCACTGGGGC CAGATGGTAA GCCCTCCCGT ATCGTAGTTA
     ATAGTAACGT CGTGACCCCG GTCTACCATT CGGGAGGGCA TAGCATCAAT

     amp
     ==================================================
3051 TCTACACGAC GGGGAGTCAG GCAACTATGG ATGAACGAAA TAGACAGATC
     AGATGTGCTG CCCCTCAGTC CGTTGATACC TACTTGCTTT ATCTGTCTAG

     amp
     ==================================
3101 GCTGAGATAG GTGCCTCACT GATTAAGCAT TGGTAACTGT CAGACCAAGT
     CGACTCTATC CACGGAGTGA CTAATTCGTA ACCATTGACA GTCTGGTTCA

3151 TTACTCATAT ATACTTTAGA TTGATTTAAA ACTTCATTTT TAATTTAAAA
     AATGAGTATA TATGAAATCT AACTAAATTT TGAAGTAAAA ATTAAATTTT

3201 GGATCTAGGT GAAGATCCTT TTTGATAATC TCATGACCAA AATCCCTTAA
     CCTAGATCCA CTTCTAGGAA AAACTATTAG AGTACTGGTT TTAGGGAATT

3251 CGTGAGTTTT CGTTCCACTG AGCGTCAGAC CCCGTAGAAA AGATCAAAGG
     GCACTCAAAA GCAAGGTGAC TCGCAGTCTG GGGCATCTTT TCTAGTTTCC

3301 ATCTTCTTGA GATCCTTTTT TTCTGCGCGT AATCTGCTGC TTGCAAACAA
     TAGAAGAACT CTAGGAAAAA AAGACGCGCA TTAGACGACG AACGTTTGTT

3351 AAAAACCACC GCTACCAGCG GTGGTTTGTT TGCCGGATCA AGAGCTACCA
     TTTTTGGTGG CGATGGTCGC CACCAAACAA ACGGCCTAGT TCTCGATGGT

3401 ACTCTTTTTC CGAAGGTAAC TGGCTTCAGC AGAGCGCAGA TACCAAATAC
     TGAGAAAAAG GCTTCCATTG ACCGAAGTCG TCTCGCGTCT ATGGTTTATG
```

Fig. 11 continued

```
3451 TGTCCTTCTA GTGTAGCCGT AGTTAGGCCA CCACTTCAAG AACTCTGTAG
     ACAGGAAGAT CACATCGGCA TCAATCCGGT GGTGAAGTTC TTGAGACATC

3501 CACCGCCTAC ATACCTCGCT CTGCTAATCC TGTTACCAGT GGCTGCTGCC
     GTGGCGGATG TATGGAGCGA GACGATTAGG ACAATGGTCA CCGACGACGG

3551 AGTGGCGATA AGTCGTGTCT TACCGGGTTG GACTCAAGAC GATAGTTACC
     TCACCGCTAT TCAGCACAGA ATGGCCCAAC CTGAGTTCTG CTATCAATGG

3601 GGATAAGGCG CAGCGGTCGG GCTGAACGGG GGGTTCGTGC ACACAGCCCA
     CCTATTCCGC GTCGCCAGCC CGACTTGCCC CCCAAGCACG TGTGTCGGGT

3651 GCTTGGAGCG AACGACCTAC ACCGAACTGA GATACCTACA GCGTGAGCAT
     CGAACCTCGC TTGCTGGATG TGGCTTGACT CTATGGATGT CGCACTCGTA

3701 TGAGAAAGCG CCACGCTTCC CGAAGGGAGA AAGGCGGACA GGTATCCGGT
     ACTCTTTCGC GGTGCGAAGG GCTTCCCTCT TTCCGCCTGT CCATAGGCCA

3751 AAGCGGCAGG GTCGGAACAG GAGAGCGCAC GAGGGAGCTT CCAGGGGGAA
     TTCGCCGTCC CAGCCTTGTC CTCTCGCGTG CTCCCTCGAA GGTCCCCCTT

3801 ACGCCTGGTA TCTTTATAGT CCTGTCGGGT TTCGCCACCT CTGACTTGAG
     TGCGGACCAT AGAAATATCA GGACAGCCCA AAGCGGTGGA GACTGAACTC

3851 CGTCGATTTT TGTGATGCTC GTCAGGGGGG CGGAGCCTAT GGAAAAACGC
     GCAGCTAAAA ACACTACGAG CAGTCCCCCC GCCTCGGATA CCTTTTTGCG

3901 CAGCAACGCG GCCTTTTTAC GGTTCCTGGC CTTTTGCTGG CCTTTTGCTC
     GTCGTTGCGC CGGAAAAATG CCAAGGACCG GAAAACGACC GGAAAACGAG

3951 ACATGTTCTT TCCTGCGTTA TCCCCTGATT CTGTGGATAA CCGTATTACC
     TGTACAAGAA AGGACGCAAT AGGGGACTAA GACACCTATT GGCATAATGG

4001 GCCTTTGAGT GAGCTGATAC CGCTCGCCGC AGCCGAACGA CCGAGCGCAG
     CGGAAACTCA CTCGACTATG GCGAGCGGCG TCGGCTTGCT GGCTCGCGTC

4051 CGAGTCAGTG AGCGAGGAAG CGGAAGAGCG CCCAATACGC AAACCGCCTC
     GCTCAGTCAC TCGCTCCTTC GCCTTCTCGC GGGTTATGCG TTTGGCGGAG

4101 TCCCCGCGCG TTGGCCGATT CATTAATGCA GCTGGCACGA CAGGTTTCCC
     AGGGGCGCGC AACCGGCTAA GTAATTACGT CGACCGTGCT GTCCAAAGGG

4151 GACTGGAAAG CGGGCAGTGA GCGCAACGCA ATTAATGTGA GTTAGCTCAC
     CTGACCTTTC GCCCGTCACT CGCGTTGCGT TAATTACACT CAATCGAGTG

4201 TCATTAGGCA CCCCAGGCTT TACACTTTAT GCTTCCGGCT CGTATGTTGT
     AGTAATCCGT GGGGTCCGAA ATGTGAAATA CGAAGGCCGA GCATACAACA

4251 GTGGAATTGT GAGCGGATAA CAATTTCACA CAGGAAACAG CT
     CACCTTAACA CTCGCCTATT GTTAAAGTGT GTCCTTTGTC GA
```

Fig. 12

II. Predicted DNA sequence pGQ1

```
                                  ctl-1 promoter  + coding  region
                                                                 =
                                                             o-GQ1
                                                                 =
                                              PstI
                                            ~~~~~~~
  1   ATGACCATGA TTACGCCAAG CTTGCATGCC TGCAGCCAAT GCATTGGAAG
      TACTGGTACT AATGCGGTTC GAACGTACGG ACGTCGGTTA CGTAACCTTC

                                  ctl-1 promoter  + coding  region
      ==================================================
                    o-GQ1
      ==================================
 51   AGATATTTTG CGCGTCAAAT ATGTTTTGTG TCCCCGTAAT ATTTTTTTAA
      TCTATAAAAC GCGCAGTTTA TACAAAACAC AGGGGCATTA TAAAAAAATT

                                  ctl-1 promoter  + coding  region
      ==================================================
101   ATCAAATTTC ACATTTTAAC CATAAAAAAC TCTTTCAAAA GTGTAATTTT
      TAGTTTAAAG TGTAAAATTG GTATTTTTTG AGAAAGTTTT CACATTAAAA

                                  ctl-1 promoter  + coding  region
      ==================================================
151   CTACGCAAAA ATGCCGTTCG GATGAAAAAT TACTTTTGAA AAACAAACTC
      GATGCGTTTT TACGGCAAGC CTACTTTTTA ATGAAAACTT TTTGTTTGAG

                                  ctl-1 promoter  + coding  region
      ==================================================
201   GAAACTACGG TACGCAAAAA AGTACATCGG TGTTTGCACA TAAGTGAAAA
      CTTTGATGCC ATGCGTTTTT TCATGTAGCC ACAAACGTGT ATTCACTTTT

                                  ctl-1 promoter  + coding  region
      ==================================================
251   CAATGTTGTT TTTTTGTAAT TAAAATCGAT TAATTTTTTT TCCCGGAAAA
      GTTACAACAA AAAAACATTA ATTTTAGCTA ATTAAAAAAA AGGGCCTTTT

                                  ctl-1 promoter  + coding  region
      ==================================================
301   CAAAAACGTT TTCAGCGTGG ATTTCTATTG TTTCTTGCGT AAAAAAAAAT
      GTTTTTGCAA AAGTCGCACC TAAAGATAAC AAAGAACGCA TTTTTTTTTA

                                  ctl-1 promoter  + coding  region
      ==================================================
351   TATTTACCAA TTTTAAACGA TAATTTCCAC GAATTTTCGC CATTAATCTC
      ATAAATGGTT AAAATTTGCT ATTAAAGGTG CTTAAAAGCG GTAATTAGAG

                                  ctl-1 promoter  + coding  region
      ==================================================
401   TCGATTTTGT TGATTCTTGA CTCCGAGCAA TCTCTCCGGT TTTCGCAAAC
      AGCTAAAACA ACTAAGAACT GAGGCTCGTT AGAGAGGCCA AAAGCGTTTG
```

Fig. 12 continued

```
                                ctl-1 promoter  + coding  region
    ======================================================
451 GATTATATTA TTTATTTGTT TTCCTTTTCA GTGCCGATTC TCGGAAATTC
    CTAATATAAT AAATAAACAA AAGGAAAAGT CACGGCTAAG AGCCTTTAAG

                                ctl-1 promoter  + coding  region
    ======================================================
                                        Exon  1
                      ==================================
501 AACAGTAAAT CTTCAAAATG CCAATGCTTC CCCACATGGT CAATCTAAGT
    TTGTCATTTA GAAGTTTTAC GGTTACGAAG GGGTGTACCA GTTAGATTCA

                                ctl-1 promoter  + coding  region
    ======================================================
551 GAGTTTCTTT GTTACAAAAT ACACGTGATG TCAGATTGTC TCATTTCGGT
    CTCAAAGAAA CAATGTTTTA TGTGCACTAC AGTCTAACAG AGTAAAGCCA

                      ctl-1 promoter  + coding  region
    ======================================================
601 TTGATCTACG TAGATCTACA AAAAATGCGG GAATTGAGCC GCAGAGTTCT
    AACTAGATGC ATCTAGATGT TTTTTACGCC CTTAACTCGG CGTCTCAAGA

    ctl-1 promoter  + coding  region
    ======================================================
651 CAACTGCTTT CGCATGGTTA AGAACGTGCG GACGTCAAAT TGTTTTGGGC
    GTTGACGAAA GCGTACCAAT TCTTGCACGC CTGCAGTTTA ACAAAACCCG

    ctl-1 promoter  + coding  region
    ======================================================
701 AAAAATTCCC GCATTTTTTG TAGATCAAAC CGTAATGGGA CAGTCTGGCA
    TTTTTAAGGG CGTAAAAAAC ATCTAGTTTG GCATTACCCT GTCAGACCGT

    ctl-1 promoter  + coding  region
    ======================================================
                                                  Exon 2
                                ============================
751 CCACGTGACT ATATATTTTT AGCGGTCAAC GACACAAAAC CCGGACCAAT
    GGTGCACTGA TATATAAAAA TCGCCAGTTG CTGTGTTTTG GGCCTGGTTA

    ctl-1 promoter  + coding  region
    ======================================================
       Exon 2
    ==========================================
801 GGCTGAGGAT CAGCTGAAAG CTTATAGAGA TAGAAATCAG GTGAGAAAAA
    CCGACTCCTA GTCGACTTTC GAATATCTCT ATCTTTAGTC CACTCTTTTT

    ctl-1 promoter  + coding  region
    ======================================================
851 TCAATTTCAG CGATTTTCTT CGCAATTTAT ATAAAAACTG ATTTTTCCAG
    AGTTAAAGTC GCTAAAAGAA GCGTTAAATA TATTTTTGAC TAAAAAGGTC

    ctl-1 promoter  + coding  region
    ======================================================
                                             Exon  3 partial
```

Fig. 12 continued

```
     ==================================================
901  GAACCCCACC TGCTCACCAC ATCCAATGGA GCTCCGATCT ACTCGAAGAC
     CTTGGGGTGG ACGAGTGGTG TAGGTTACCT CGAGGCTAGA TGAGCTTCTG

     ctl-1 promoter  + coding  region
     ==================================================
                                         Exon  3 partial
     ==================================================
951  CGCCGTGCTC ACCGCCGGAC GACGTGGTCC AATGCTAATG CAGGACATCG
     GCGGCACGAG TGGCGGCCTG CTGCACCAGG TTACGATTAC GTCCTGTAGC

     ctl-1 promoter  + coding  region
     ==================================================
                                         Exon  3 partial
     ==================================================
1001 TTTATATGGA CGAGATGGCT CATTTCGATC GTGAACGCAT CCCGGAGCGT
     AAATATACCT GCTCTACCGA GTAAAGCTAG CACTTGCGTA GGGCCTCGCA

     ctl-1 promoter  + coding  region
     ==================================================
     Exon 3 partial
     ==================================================
1051 GTCGTCCATG CCAAAGGTGG TGGTGCTCAT GGATACTTCG AGGTCACCCA
     CAGCAGGTAC GGTTTCCACC ACCACGAGTA CCTATGAAGC TCCAGTGGGT

     ctl-1 promoter  + coding  region
     ==================================================
     Exon 3 partial
     ==================================================
1101 TGACATCACC AAGTACTGTA AGGCCGATAT GTTCAACAAG GTCGGAAAAC
     ACTGTAGTGG TTCATGACAT TCCGGCTATA CAAGTTGTTC CAGCCTTTTG

     ctl-1 promoter  + coding  region
     ==================================================
                                          o-GQ2bis
                                  =====================
     Exon 3 partial
     ==================================================
                                                   BamHI
                                                     ~~~
1151 AGACACCACT TCTCGTTCGT TTTTCAACGG TCGCTGGAGA ATCGGCCGGA
     TCTGTGGTGA AGAGCAAGCA AAAAGTTGCC AGCGACCTCT TAGCCGGCCT

     ctl-1 promoter  + coding  region
     ===
     o-GQ2bis
     ===
     Exon 3 partial                       synth.  intron A
     ===                           ======================
     BamHI
     ~~~
1201 TCCCCGGGAT TGGCCAAAGG ACCCAAAGGT ATGTTTCGAA TGATACTAAC
     AGGGGCCCTA ACCGGTTTCC TGGGTTTCCA TACAAAGCTT ACTATGATTG
```

Fig. 12 continued

```
     synth. intron  A
     ====================
1251 ATAACATAGA ACATTTTCAG GAGGACCCTT GGCTAGCGTC GACGGTACCA
     TATTGTATCT TGTAAAAGTC CTCCTGGGAA CCGATCGCAG CTGCCATGGT

                                                      GFPI
                ========================================
1301 TGGGGCGCGC CATGAGTAAA GGAGAAGAAC TTTTCACTGG AGTTGTCCCA
     ACCCCGCGCG GTACTCATTT CCTCTTCTTG AAAAGTGACC TCAACAGGGT

                                                     GFPI
     ==================================================
1351 ATTCTTGTTG AATTAGATGG TGATGTTAAT GGGCACAAAT TTTCTGTCAG
     TAAGAACAAC TTAATCTACC ACTACAATTA CCCGTGTTTA AAAGACAGTC

     GFPI
     ==================================================
1401 TGGAGAGGGT GAAGGTGATG CAACATACGG AAAACTTACC CTTAAATTTA
     ACCTCTCCCA CTTCCACTAC GTTGTATGCC TTTTGAATGG GAATTTAAAT

     GFPI
     ================================
1451 TTTGCACTAC TGGAAAACTA CCTGTTCCAT GGGTAAGTTT AAACATATAT
     AAACGTGATG ACCTTTTGAT GGACAAGGTA CCCATTCAAA TTTGTATATA

                                                     GFPII
                                      =================
1501 ATACTAACTA ACCCTGATTA TTTAAATTTT CAGCCAACAC TTGTCACTAC
     TATGATTGAT TGGGACTAAT AAATTTAAAA GTCGGTTGTG AACAGTGATG

                                                     GFPII
     ==================================================
1551 TTTCTGTTAT GGTGTTCAAT GCTTCTCGAG ATACCCAGAT CATATGAAAC
     AAAGACAATA CCACAAGTTA CGAAGAGCTC TATGGGTCTA GTATACTTTG

              GFPII
     ==================================================
1601 GGCATGACTT TTTCAAGAGT GCCATGCCCG AAGGTTATGT ACAGGAAAGA
     CCGTACTGAA AAAGTTCTCA CGGTACGGGC TTCCAATACA TGTCCTTTCT

     GFPII
     ======================================
1651 ACTATATTTT TCAAAGATGA CGGGAACTAC AAGACACGTA AGTTTAAACA
     TGATATAAAA AGTTTCTACT GCCCTTGATG TTCTGTGCAT TCAAATTTGT

                                                    GFPIII
                                                  ========
1701 GTTCGGTACT AACTAACCAT ACATATTTAA ATTTTCAGGT GCTGAAGTCA
     CAAGCCATGA TTGATTGGTA TGTATAAATT TAAAAGTCCA CGACTTCAGT

                                                    GFPIII
     ==================================================
1751 AGTTTGAAGG TGATACCCTT GTTAATAGAA TCGAGTTAAA AGGTATTGAT
     TCAAACTTCC ACTATGGGAA CAATTATCTT AGCTCAATTT TCCATAACTA
```

Fig. 12 continued

```
                         GFPIII
      ==================================================
1801  TTTAAAGAAG ATGGAAACAT TCTTGGACAC AAATTGGAAT ACAACTATAA
      AAATTTCTTC TACCTTTGTA AGAACCTGTG TTTAACCTTA TGTTGATATT

      GFPIII
      ==================================================
1851  CTCACACAAT GTATACATCA TGGCAGACAA ACAAAAGAAT GGAATCAAAG
      GAGTGTGTTA CATATGTAGT ACCGTCTGTT TGTTTTCTTA CCTTAGTTTC

      GFPIII
      ==
1901  TTGTAAGTTT AAACTTGGAC TTACTAACTA ACGGATTATA TTTAAATTTT
      AACATTCAAA TTTGAACCTG AATGATTGAT TGCCTAATAT AAATTTAAAA

                                                     GFPIV
         ===============================================
1951  CAGAACTTCA AAATTAGACA CAACATTGAA GATGGAAGCG TTCAACTAGC
      GTCTTGAAGT TTTAATCTGT GTTGTAACTT CTACCTTCGC AAGTTGATCG

                                                     GFPIV
      ==================================================
2001  AGACCATTAT CAACAAAATA CTCCAATTGG CGATGGCCCT GTCCTTTTAC
      TCTGGTAATA GTTGTTTTAT GAGGTTAACC GCTACCGGGA CAGGAAAATG

                      GFPIV
      ==================================================
2051  CAGACAACCA TTACCTGTCC ACACAATCTG CCCTTTCGAA AGATCCCAAC
      GTCTGTTGGT AATGGACAGG TGTGTTAGAC GGGAAAGCTT TCTAGGGTTG

      GFPIV
      ==================================================
2101  GAAAAGAGAG ACCACATGGT CCTTCTTGAG TTTGTAACAG CTGCTGGGAT
      CTTTTCTCTC TGGTGTACCA GGAAGAACTC AAACATTGTC GACGACCCTA

      GFPIV                              FseI
      ========================================
2151  TACACATGGC ATGGATGAAC TATACAAATA GGGCCGGCCG AGCTCCGCAT
      ATGTGTACCG TACCTACTTG ATATGTTTAT CCCGGCCGGC TCGAGGCGTA

                                            unc-54  3' UTR
                                   ===================
2201  CGGCCGCTGT CATCAGATCG CCATCTCGCG CCCGTGCCTC TGACTTCTAA
      GCCGGCGACA GTAGTCTAGC GGTAGAGCGC GGGCACGGAG ACTGAAGATT

                                            unc-54  3' UTR
      ==================================================
2251  GTCCAATTAC TCTTCAACAT CCCTACATGC TCTTTCTCCC TGTGCTCCCA
      CAGGTTAATG AGAAGTTGTA GGGATGTACG AGAAAGAGGG ACACGAGGGT

                                            unc-54  3' UTR
      ==================================================
2301  CCCCCTATTT TTGTTATTAT CAAAAAAACT TCTTCTTAAT TTCTTTGTTT
```

Fig. 12 continued

```
     GGGGGATAAA AACAATAATA GTTTTTTTGA AGAAGAATTA AAGAAACAAA

                                              unc-54  3' UTR
     ==================================================
2351 TTTAGCTTCT TTTAAGTCAC CTCTAACAAT GAAATTGTGT AGATTCAAAA
     AAATCGAAGA AAATTCAGTG GAGATTGTTA CTTTAACACA TCTAAGTTTT

                                              unc-54  3' UTR
     ==================================================
2401 ATAGAATTAA TTCGTAATAA AAAGTCGAAA AAAATTGTGC TCCCTCCCCC
     TATCTTAATT AAGCATTATT TTTCAGCTTT TTTTAACACG AGGGAGGGGG

                                              unc-54  3' UTR
     ==================================================
2451 CATTAATAAT AATTCTATCC CAAAATCTAC ACAATGTTCT GTGTACACTT
     GTAATTATTA TTAAGATAGG GTTTTAGATG TGTTACAAGA CACATGTGAA

                                              unc-54  3' UTR
     ==================================================
2501 CTTATGTTTT TTTTACTTCT GATAAATTTT TTTTGAAACA TCATAGAAAA
     GAATACAAAA AAAATGAAGA CTATTTAAAA AAAACTTTGT AGTATCTTTT

                                          unc-54 3'  UTR
     ==================================================
2551 AACCGCACAC AAAATACCTT ATCATATGTT ACGTTTCAGT TTATGACCGC
     TTGGCGTGTG TTTTATGGAA TAGTATACAA TGCAAAGTCA AATACTGGCG

     unc-54 3'  UTR
     ==================================================
2601 AATTTTTATT TCTTCGCACG TCTGGGCCTC TCATGACGTC AAATCATGCT
     TTAAAAATAA AGAAGCGTGC AGACCCGGAG AGTACTGCAG TTTAGTACGA

     unc-54 3'  UTR
     ==================================================
2651 CATCGTGAAA AAGTTTTGGA GTATTTTTGG AATTTTTCAA TCAAGTGAAA
     GTAGCACTTT TTCAAAACCT CATAAAAACC TTAAAAAGTT AGTTCACTTT

     unc-54 3'  UTR
     ==================================================
2701 GTTTATGAAA TTAATTTTCC TGCTTTTGCT TTTTGGGGGT TTCCCCTATT
     CAAATACTTT AATTAAAAGG ACGAAAACGA AAAACCCCCA AAGGGGATAA

     unc-54 3'  UTR
     ==================================================
2751 GTTTGTCAAG AGTTTCGAGG ACGGCGTTTT TCTTGCTAAA ATCACAAGTA
     CAAACAGTTC TCAAAGCTCC TGCCGCAAAA AGAACGATTT TAGTGTTCAT

     unc-54 3'  UTR
     ==================================================
2801 TTGATGAGCA CGATGCAAGA AAGATCGGAA GAAGGTTTGG GTTTGAGGCT
     AACTACTCGT GCTACGTTCT TTCTAGCCTT CTTCCAAACC CAAACTCCGA

     unc-54 3'  UTR
     ==================================================
```

Fig. 12 continued

```
2851  CAGTGGAAGG TGAGTAGAAG TTGATAATTT GAAAGTGGAG TAGTGTCTAT
      GTCACCTTCC ACTCATCTTC AACTATTAAA CTTTCACCTC ATCACAGATA

      unc-54 3'  UTR
      ==================================================
2901  GGGGTTTTTG CCTTAAATGA CAGAATACAT TCCCAATATA CCAAACATAA
      CCCCAAAAAC GGAATTTACT GTCTTATGTA AGGGTTATAT GGTTTGTATT

      unc-54 3'  UTR
      ======
2951  CTGTTTCCTA CTAGTCGGCC GTACGGGCCC TTTCGTCTCG CGCGTTTCGG
      GACAAAGGAT GATCAGCCGG CATGCCCGGG AAAGCAGAGC GCGCAAAGCC

3001  TGATGACGGT GAAAACCTCT GACACATGCA GCTCCCGGAG ACGGTCACAG
      ACTACTGCCA CTTTTGGAGA CTGTGTACGT CGAGGGCCTC TGCCAGTGTC

3051  CTTGTCTGTA AGCGGATGCC GGGAGCAGAC AAGCCCGTCA GGGCGCGTCA
      GAACAGACAT TCGCCTACGG CCCTCGTCTG TTCGGGCAGT CCCGCGCAGT

3101  GCGGGTGTTG GCGGGTGTCG GGGCTGGCTT AACTATGCGG CATCAGAGCA
      CGCCCACAAC CGCCCACAGC CCCGACCGAA TTGATACGCC GTAGTCTCGT

3151  GATTGTACTG AGAGTGCACC ATATGCGGTG TGAAATACCG CACAGATGCG
      CTAACATGAC TCTCACGTGG TATACGCCAC ACTTTATGGC GTGTCTACGC

3201  TAAGGAGAAA ATACCGCATC AGGCGGCCTT AAGGGCCTCG TGATACGCCT
      ATTCCTCTTT TATGGCGTAG TCCGCCGGAA TTCCCGGAGC ACTATGCGGA

3251  ATTTTTATAG GTTAATGTCA TGATAATAAT GGTTTCTTAG ACGTCAGGTG
      TAAAAATATC CAATTACAGT ACTATTATTA CCAAAGAATC TGCAGTCCAC

3301  GCACTTTTCG GGGAAATGTG CGCGGAACCC CTATTTGTTT ATTTTTCTAA
      CGTGAAAAGC CCCTTTACAC GCGCCTTGGG GATAAACAAA TAAAAAGATT

3351  ATACATTCAA ATATGTATCC GCTCATGAGA CAATAACCCT GATAAATGCT
      TATGTAAGTT TATACATAGG CGAGTACTCT GTTATTGGGA CTATTTACGA

                                                     amp
                                 ==========================
3401  TCAATAATAT TGAAAAAGGA AGAGTATGAG TATTCAACAT TTCCGTGTCG
      AGTTATTATA ACTTTTTCCT TCTCATACTC ATAAGTTGTA AAGGCACAGC

                                                     amp
      ==================================================
3451  CCCTTATTCC CTTTTTTGCG GCATTTTGCC TTCCTGTTTT TGCTCACCCA
      GGGAATAAGG GAAAAAACGC CGTAAAACGG AAGGACAAAA ACGAGTGGGT

                                                     amp
      ==================================================
3501  GAAACGCTGG TGAAAGTAAA AGATGCTGAA GATCAGTTGG GTGCACGAGT
      CTTTGCGACC ACTTTCATTT TCTACGACTT CTAGTCAACC CACGTGCTCA

                                                     amp
      ==================================================
```

Fig. 12 continued

```
3551  GGGTTACATC GAACTGGATC TCAACAGCGG TAAGATCCTT GAGAGTTTTC
      CCCAATGTAG CTTGACCTAG AGTTGTCGCC ATTCTAGGAA CTCTCAAAAG

                                                       amp
      ==================================================
3601  GCCCCGAAGA ACGTTTTCCA ATGATGAGCA CTTTTAAAGT TCTGCTATGT
      CGGGGCTTCT TGCAAAAGGT TACTACTCGT GAAAATTTCA AGACGATACA

                                                       amp
      ==================================================
3651  GGCGCGGTAT TATCCCGTAT TGACGCCGGG CAAGAGCAAC TCGGTCGCCG
      CCGCGCCATA ATAGGGCATA ACTGCGGCCC GTTCTCGTTG AGCCAGCGGC

                                                       amp
      ==================================================
3701  CATACACTAT TCTCAGAATG ACTTGGTTGA GTACTCACCA GTCACAGAAA
      GTATGTGATA AGAGTCTTAC TGAACCAACT CATGAGTGGT CAGTGTCTTT

                                                       amp
      ==================================================
3751  AGCATCTTAC GGATGGCATG ACAGTAAGAG AATTATGCAG TGCTGCCATA
      TCGTAGAATG CCTACCGTAC TGTCATTCTC TTAATACGTC ACGACGGTAT

                                                       amp
      ==================================================
3801  ACCATGAGTG ATAACACTGC GGCCAACTTA CTTCTGACAA CGATCGGAGG
      TGGTACTCAC TATTGTGACG CCGGTTGAAT GAAGACTGTT GCTAGCCTCC

         amp
      ==================================================
3851  ACCGAAGGAG CTAACCGCTT TTTTGCACAA CATGGGGGAT CATGTAACTC
      TGGCTTCCTC GATTGGCGAA AAAACGTGTT GTACCCCCTA GTACATTGAG

      amp
      ==================================================
3901  GCCTTGATCG TTGGGAACCG GAGCTGAATG AAGCCATACC AAACGACGAG
      CGGAACTAGC AACCCTTGGC CTCGACTTAC TTCGGTATGG TTTGCTGCTC

      amp
      ==================================================
3951  CGTGACACCA CGATGCCTGT AGCAATGGCA ACAACGTTGC GCAAACTATT
      GCACTGTGGT GCTACGGACA TCGTTACCGT TGTTGCAACG CGTTTGATAA

      amp
      ==================================================
4001  AACTGGCGAA CTACTTACTC TAGCTTCCCG GCAACAATTA ATAGACTGGA
      TTGACCGCTT GATGAATGAG ATCGAAGGGC CGTTGTTAAT TATCTGACCT

      amp
      ==================================================
4051  TGGAGGCGGA TAAAGTTGCA GGACCACTTC TGCGCTCGGC CCTTCCGGCT
      ACCTCCGCCT ATTTCAACGT CCTGGTGAAG ACGCGAGCCG GGAAGGCCGA

      amp
```

Fig. 12 continued

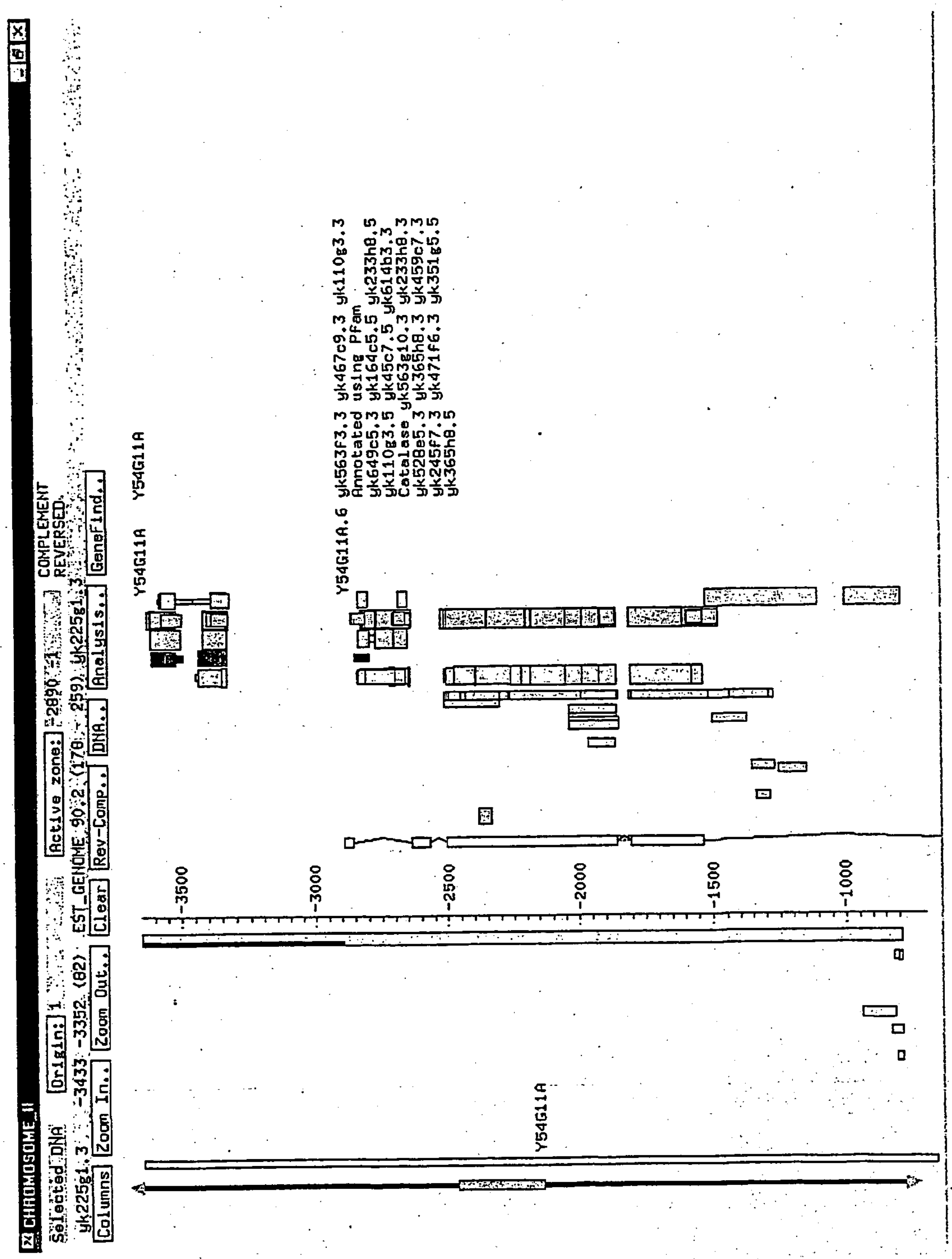
```
          ==================================================
4101      GGCTGGTTTA TTGCTGATAA ATCTGGAGCC GGTGAGCGTG GGTCTCGCGG
          CCGACCAAAT AACGACTATT TAGACCTCGG CCACTCGCAC CCAGAGCGCC

          amp
          ==================================================
4151      TATCATTGCA GCACTGGGGC CAGATGGTAA GCCCTCCCGT ATCGTAGTTA
          ATAGTAACGT CGTGACCCCG GTCTACCATT CGGGAGGGCA TAGCATCAAT

          amp
          ==================================================
4201      TCTACACGAC GGGGAGTCAG GCAACTATGG ATGAACGAAA TAGACAGATC
          AGATGTGCTG CCCCTCAGTC CGTTGATACC TACTTGCTTT ATCTGTCTAG

          amp
          ==================================
4251      GCTGAGATAG GTGCCTCACT GATTAAGCAT TGGTAACTGT CAGACCAAGT
          CGACTCTATC CACGGAGTGA CTAATTCGTA ACCATTGACA GTCTGGTTCA

4301      TTACTCATAT ATACTTTAGA TTGATTTAAA ACTTCATTTT TAATTTAAAA
          AATGAGTATA TATGAAATCT AACTAAATTT TGAAGTAAAA ATTAAATTTT

4351      GGATCTAGGT GAAGATCCTT TTTGATAATC TCATGACCAA AATCCCTTAA
          CCTAGATCCA CTTCTAGGAA AAACTATTAG AGTACTGGTT TTAGGGAATT

4401      CGTGAGTTTT CGTTCCACTG AGCGTCAGAC CCCGTAGAAA AGATCAAAGG
          GCACTCAAAA GCAAGGTGAC TCGCAGTCTG GGGCATCTTT TCTAGTTTCC

4451      ATCTTCTTGA GATCCTTTTT TTCTGCGCGT AATCTGCTGC TTGCAAACAA
          TAGAAGAACT CTAGGAAAAA AAGACGCGCA TTAGACGACG AACGTTTGTT

4501      AAAAAACCACC GCTACCAGCG GTGGTTTGTT TGCCGGATCA AGAGCTACCA
          TTTTTGGTGG CGATGGTCGC CACCAAACAA ACGGCCTAGT TCTCGATGGT

4551      ACTCTTTTTC CGAAGGTAAC TGGCTTCAGC AGAGCGCAGA TACCAAATAC
          TGAGAAAAAG GCTTCCATTG ACCGAAGTCG TCTCGCGTCT ATGGTTTATG

4601      TGTCCTTCTA GTGTAGCCGT AGTTAGGCCA CCACTTCAAG AACTCTGTAG
          ACAGGAAGAT CACATCGGCA TCAATCCGGT GGTGAAGTTC TTGAGACATC

4651      CACCGCCTAC ATACCTCGCT CTGCTAATCC TGTTACCAGT GGCTGCTGCC
          GTGGCGGATG TATGGAGCGA GACGATTAGG ACAATGGTCA CCGACGACGG

4701      AGTGGCGATA AGTCGTGTCT TACCGGGTTG GACTCAAGAC GATAGTTACC
          TCACCGCTAT TCAGCACAGA ATGGCCCAAC CTGAGTTCTG CTATCAATGG

4751      GGATAAGGCG CAGCGGTCGG GCTGAACGGG GGGTTCGTGC ACACAGCCCA
          CCTATTCCGC GTCGCCAGCC CGACTTGCCC CCCAAGCACG TGTGTCGGGT

4801      GCTTGGAGCG AACGACCTAC ACCGAACTGA GATACCTACA GCGTGAGCAT
          CGAACCTCGC TTGCTGGATG TGGCTTGACT CTATGGATGT CGCACTCGTA

4851      TGAGAAAGCG CCACGCTTCC CGAAGGGAGA AAGGCGGACA GGTATCCGGT
          ACTCTTTCGC GGTGCGAAGG GCTTCCCTCT TTCCGCCTGT CCATAGGCCA
```

Fig. 12 continued

```
4901  AAGCGGCAGG GTCGGAACAG GAGAGCGCAC GAGGGAGCTT CCAGGGGGAA
      TTCGCCGTCC CAGCCTTGTC CTCTCGCGTG CTCCCTCGAA GGTCCCCCTT

4951  ACGCCTGGTA TCTTTATAGT CCTGTCGGGT TTCGCCACCT CTGACTTGAG
      TGCGGACCAT AGAAATATCA GGACAGCCCA AAGCGGTGGA GACTGAACTC

5001  CGTCGATTTT TGTGATGCTC GTCAGGGGGG CGGAGCCTAT GGAAAAACGC
      GCAGCTAAAA ACACTACGAG CAGTCCCCCC GCCTCGGATA CCTTTTTGCG

5051  CAGCAACGCG GCCTTTTTAC GGTTCCTGGC CTTTTGCTGG CCTTTTGCTC
      GTCGTTGCGC CGGAAAAATG CCAAGGACCG GAAAACGACC GGAAAACGAG

5101  ACATGTTCTT TCCTGCGTTA TCCCCTGATT CTGTGGATAA CCGTATTACC
      TGTACAAGAA AGGACGCAAT AGGGGACTAA GACACCTATT GGCATAATGG

5151  GCCTTTGAGT GAGCTGATAC CGCTCGCCGC AGCCGAACGA CCGAGCGCAG
      CGGAAACTCA CTCGACTATG GCGAGCGGCG TCGGCTTGCT GGCTCGCGTC

5201  CGAGTCAGTG AGCGAGGAAG CGGAAGAGCG CCCAATACGC AAACCGCCTC
      GCTCAGTCAC TCGCTCCTTC GCCTTCTCGC GGGTTATGCG TTTGGCGGAG

5251  TCCCCGCGCG TTGGCCGATT CATTAATGCA GCTGGCACGA CAGGTTTCCC
      AGGGGCGCGC AACCGGCTAA GTAATTACGT CGACCGTGCT GTCCAAAGGG

5301  GACTGGAAAG CGGGCAGTGA GCGCAACGCA ATTAATGTGA GTTAGCTCAC
      CTGACCTTTC GCCCGTCACT CGCGTTGCGT TAATTACACT CAATCGAGTG

5351  TCATTAGGCA CCCCAGGCTT TACACTTTAT GCTTCCGGCT CGTATGTTGT
      AGTAATCCGT GGGGTCCGAA ATGTGAAATA CGAAGGCCGA GCATACAACA

5401  GTGGAATTGT GAGCGGATAA CAATTTCACA CAGGAAACAG CT
      CACCTTAACA CTCGCCTATT GTTAAAGTGT GTCCTTTGTC GA
```

Fig. 13

ctl-1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Selected DNA
Origin: 1
Active zone:
COMPLEMENT
REVERSED
Columns
Zoom In..
Zoom Out..
Clear
Rev-Comp..
DNA..
Analysis..
GeneFind..
Y54G11A
Y54G11A.6
Annotated using Pfam
Catalase
-3500
-3000
-2500
-2000
-1500
-1000

Figure 14

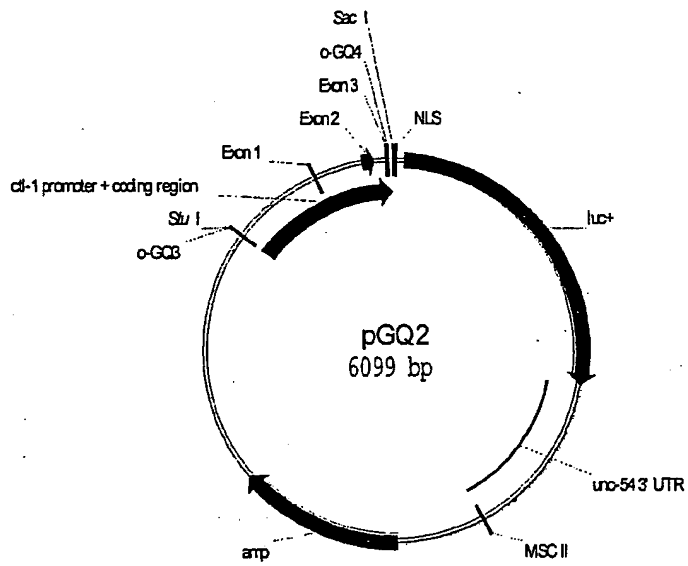


Figure 15

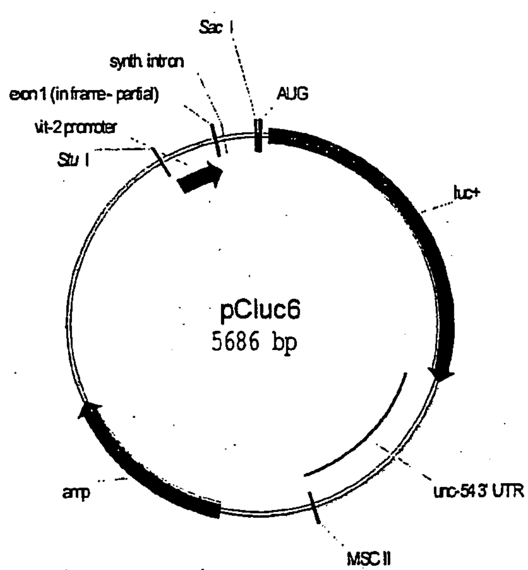


Fig. 16

pCluc6 sequence:

```
     AUG                                          luc+
     ===                                      ========
  1  ATGACTGCTC CAAAGAAGAA GCGTAAGGTA CCGGTAGAAA AAATGGAAGA
     TACTGACGAG GTTTCTTCTT CGCATTCCAT GGCCATCTTT TTTACCTTCT

                                                  luc+
     ==================================================
 51  CGCCAAAAAC ATAAAGAAAG GCCCGGCGCC ATTCTATCCG CTGGAAGATG
     GCGGTTTTTG TATTTCTTTC CGGGCCGCGG TAAGATAGGC GACCTTCTAC

                                                  luc+
     ==================================================
101  GAACCGCTGG AGAGCAACTG CATAAGGCTA TGAAGAGATA CGCCCTGGTT
     CTTGGCGACC TCTCGTTGAC GTATTCCGAT ACTTCTCTAT GCGGGACCAA

                                                  luc+
     ==================================================
151  CCTGGAACAA TTGCTTTTAC AGATGCACAT ATCGAGGTGG ACATCACTTA
     GGACCTTGTT AACGAAAATG TCTACGTGTA TAGCTCCACC TGTAGTGAAT

                                                  luc+
     ==================================================
201  CGCTGAGTAC TTCGAAATGT CCGTTCGGTT GGCAGAAGCT ATGAAACGAT
     GCGACTCATG AAGCTTTACA GGCAAGCCAA CCGTCTTCGA TACTTTGCTA

                                                  luc+
     ==================================================
251  ATGGGCTGAA TACAAATCAC AGAATCGTCG TATGCAGTGA AAACTCTCTT
     TACCCGACTT ATGTTTAGTG TCTTAGCAGC ATACGTCACT TTTGAGAGAA

                                                  luc+
     ==================================================
301  CAATTCTTTA TGCCGGTGTT GGGCGCGTTA TTTATCGGAG TTGCAGTTGC
     GTTAAGAAAT ACGGCCACAA CCCGCGCAAT AAATAGCCTC AACGTCAACG

                                                  luc+
     ==================================================
351  GCCCGCGAAC GACATTTATA ATGAACGTGA ATTGCTCAAC AGTATGGGCA
     CGGGCGCTTG CTGTAAATAT TACTTGCACT TAACGAGTTG TCATACCCGT

                                                  luc+
     ==================================================
401  TTTCGCAGCC TACCGTGGTG TTCGTTTCCA AAAAGGGGTT GCAAAAAATT
     AAAGCGTCGG ATGGCACCAC AAGCAAAGGT TTTTCCCCAA CGTTTTTTAA

                                                  luc+
     ==================================================
451  TTGAACGTGC AAAAAAAGCT CCCAATCATC CAAAAAATTA TTATCATGGA
     AACTTGCACG TTTTTTTCGA GGGTTAGTAG GTTTTTTAAT AATAGTACCT

                                                  luc+
```

Fig. 16 continued

```
     ==================================================
501  TTCTAAAACG GATTACCAGG GATTTCAGTC GATGTACACG TTCGTCACAT
     AAGATTTTGC CTAATGGTCC CTAAAGTCAG CTACATGTGC AAGCAGTGTA

                                                      luc+
     ==================================================
551  CTCATCTACC TCCCGGTTTT AATGAATACG ATTTTGTGCC AGAGTCCTTC
     GAGTAGATGG AGGGCCAAAA TTACTTATGC TAAAACACGG TCTCAGGAAG

                                                      luc+
     ==================================================
601  GATAGGGACA AGACAATTGC ACTGATCATG AACTCCTCTG GATCTACTGG
     CTATCCCTGT TCTGTTAACG TGACTAGTAC TTGAGGAGAC CTAGATGACC

                                                      luc+
     ==================================================
651  TCTGCCTAAA GGTGTCGCTC TGCCTCATAG AACTGCCTGC GTGAGATTCT
     AGACGGATTT CCACAGCGAG ACGGAGTATC TTGACGGACG CACTCTAAGA

                                                      luc+
     ==================================================
701  CGCATGCCAG AGATCCTATT TTTGGCAATC AAATCATTCC GGATACTGCG
     GCGTACGGTC TCTAGGATAA AAACCGTTAG TTTAGTAAGG CCTATGACGC

                                                      luc+
     ==================================================
751  ATTTAAGTG TTGTTCCATT CCATCACGGT TTTGGAATGT TTACTACACT
     TAAAATTCAC AACAAGGTAA GGTAGTGCCA AAACCTTACA AATGATGTGA

                                                      luc+
     ==================================================
801  CGGATATTTG ATATGTGGAT TTCGAGTCGT CTTAATGTAT AGATTTGAAG
     GCCTATAAAC TATACACCTA AAGCTCAGCA GAATTACATA TCTAAACTTC

                        luc+
     ==================================================
851  AAGAGCTGTT TCTGAGGAGC CTTCAGGATT ACAAGATTCA AAGTGCGCTG
     TTCTCGACAA AGACTCCTCG GAAGTCCTAA TGTTCTAAGT TTCACGCGAC

     luc+
     ==================================================
901  CTGGTGCCAA CCCTATTCTC CTTCTTCGCC AAAAGCACTC TGATTGACAA
     GACCACGGTT GGGATAAGAG GAAGAAGCGG TTTTCGTGAG ACTAACTGTT

     luc+
     ==================================================
951  ATACGATTTA TCTAATTTAC ACGAAATTGC TTCTGGTGGC GCTCCCCTCT
     TATGCTAAAT AGATTAAATG TGCTTTAACG AAGACCACCG CGAGGGGAGA

     luc+
     ==================================================
1001 CTAAGGAAGT CGGGGAAGCG GTTGCCAAGA GGTTCCATCT GCCAGGTATC
     GATTCCTTCA GCCCCTTCGC CAACGGTTCT CCAAGGTAGA CGGTCCATAG
```

Fig. 16 continued

```
     luc+
     ==================================================
1051 AGGCAAGGAT ATGGGCTCAC TGAGACTACA TCAGCTATTC TGATTACACC
     TCCGTTCCTA TACCCGAGTG ACTCTGATGT AGTCGATAAG ACTAATGTGG

     luc+
     ==================================================
1101 CGAGGGGGAT GATAAACCGG GCGCGGTCGG TAAAGTTGTT CCATTTTTTG
     GCTCCCCCTA CTATTTGGCC CGCGCCAGCC ATTTCAACAA GGTAAAAAAC

     luc+
     ==================================================
1151 AAGCGAAGGT TGTGGATCTG GATACCGGGA AAACGCTGGG CGTTAATCAA
     TTCGCTTCCA ACACCTAGAC CTATGGCCCT TTTGCGACCC GCAATTAGTT

     luc+
     ==================================================
1201 AGAGGCGAAC TGTGTGTGAG AGGTCCTATG ATTATGTCCG GTTATGTAAA
     TCTCCGCTTG ACACACACTC TCCAGGATAC TAATACAGGC CAATACATTT

     luc+
     ==================================================
1251 CAATCCGGAA GCGACCAACG CCTTGATTGA CAAGGATGGA TGGCTACATT
     GTTAGGCCTT CGCTGGTTGC GGAACTAACT GTTCCTACCT ACCGATGTAA

     luc+
     ==================================================
1301 CTGGAGACAT AGCTTACTGG GACGAAGACG AACACTTCTT CATCGTTGAC
     GACCTCTGTA TCGAATGACC CTGCTTCTGC TTGTGAAGAA GTAGCAACTG

     luc+
     ==================================================
1351 CGCCTGAAGT CTCTGATTAA GTACAAAGGC TATCAGGTGG CTCCCGCTGA
     GCGGACTTCA GAGACTAATT CATGTTTCCG ATAGTCCACC GAGGGCGACT

     luc+
     ==================================================
1401 ATTGGAATCC ATCTTGCTCC AACACCCCAA CATCTTCGAC GCAGGTGTCG
     TAACCTTAGG TAGAACGAGG TTGTGGGGTT GTAGAAGCTG CGTCCACAGC

     luc+
     ==================================================
1451 CAGGTCTTCC CGACGATGAC GCCGGTGAAC TTCCCGCCGC CGTTGTTGTT
     GTCCAGAAGG GCTGCTACTG CGGCCACTTG AAGGGCGGCG GCAACAACAA

     luc+
     ==================================================
1501 TTGGAGCACG GAAAGACGAT GACGGAAAAA GAGATCGTGG ATTACGTCGC
     AACCTCGTGC CTTTCTGCTA CTGCCTTTTT CTCTAGCACC TAATGCAGCG

     luc+
     ==================================================
1551 CAGTCAAGTA ACAACCGCGA AAAAGTTGCG CGGAGGAGTT GTGTTTGTGG
     GTCAGTTCAT TGTTGGCGCT TTTTCAACGC GCCTCCTCAA CACAAACACC
```

Fig. 16 continued

```
        luc+
        ==============================----------------------
1601    ACGAAGTACC GAAAGGTCTT ACCGGAAAAC TCGACGCAAG AAAAATCAGA
        TGCTTCATGG CTTTCCAGAA TGGCCTTTTG AGCTGCGTTC TTTTTAGTCT

        luc+
        ================================================
1651    GAGATCCTCA TAAAGGCCAA GAAGGGCGGA AAGATCGCCG TGTAATTCTA
        CTCTAGGAGT ATTTCCGGTT CTTCCCGCCT TTCTAGCGGC ACATTAAGAT

                                                unc-54  3' UTR
                   ==========================================
1701    GGAATTCCAA CTGAGCGCCG GTCGCTACCA TTACCAACTT GTCTGGTGTC
        CCTTAAGGTT GACTCGCGGC CAGCGATGGT AATGGTTGAA CAGACCACAG

                                                unc-54  3' UTR
        =====================================================
1751    AAAAATAATA GGGGCCGCTG TCATCAGAGT AAGTTTAAAC TGAGTTCTAC
        TTTTTATTAT CCCCGGCGAC AGTAGTCTCA TTCAAATTTG ACTCAAGATG

                                                unc-54  3' UTR
        =====================================================
1801    TAACTAACGA GTAATATTTA AATTTTCAGC ATCTCGCGCC CGTGCCTCTG
        ATTGATTGCT CATTATAAAT TTAAAAGTCG TAGAGCGCGG GCACGGAGAC

                                                unc-54  3' UTR
        =====================================================
1851    ACTTCTAAGT CCAATTACTC TTCAACATCC CTACATGCTC TTTCTCCCTG
        TGAAGATTCA GGTTAATGAG AAGTTGTAGG GATGTACGAG AAAGAGGGAC

                                                unc-54  3' UTR
        =====================================================
1901    TGCTCCCACC CCCTATTTTT GTTATTATCA AAAAAACTTC TTCTTAATTT
        ACGAGGGTGG GGGATAAAAA CAATAATAGT TTTTTTGAAG AAGAATTAAA

                                                unc-54  3' UTR
        =====================================================
1951    CTTTGTTTTT TAGCTTCTTT TAAGTCACCT CTAACAATGA AATTGTGTAG
        GAAACAAAAA ATCGAAGAAA ATTCAGTGGA GATTGTTACT TTAACACATC

                                                unc-54  3' UTR
        =====================================================
2001    ATTCAAAAAT AGAATTAATT CGTAATAAAA AGTCGAAAAA AATTGTGCTC
        TAAGTTTTTA TCTTAATTAA GCATTATTTT TCAGCTTTTT TTAACACGAG

                                                unc-54  3' UTR
        =====================================================
2051    CCTCCCCCCA TTAATAATAA TTCTATCCCA AAATCTACAC AATGTTCTGT
        GGAGGGGGGT AATTATTATT AAGATAGGGT TTTAGATGTG TTACAAGACA

                                    unc-54  3' UTR
        =====================================================
2101    GTACACTTCT TATGTTTTTT TTACTTCTGA TAAATTTTTT TTGAAACATC
```

Fig. 16 continued

```
      CATGTGAAGA ATACAAAAAA AATGAAGACT ATTTAAAAAA AACTTTGTAG

      unc-54 3'  UTR
      ==================================================
2151  ATAGAAAAAA CCGCACACAA AATACCTTAT CATATGTTAC GTTTCAGTTT
      TATCTTTTTT GGCGTGTGTT TTATGGAATA GTATACAATG CAAAGTCAAA

      unc-54 3'  UTR
      ==================================================
2201  ATGACCGCAA TTTTTATTTC TTCGCACGTC TGGGCCTCTC ATGACGTCAA
      TACTGGCGTT AAAAATAAAG AAGCGTGCAG ACCCGGAGAG TACTGCAGTT

      unc-54 3'  UTR
      ==================================================
2251  ATCATGCTCA TCGTGAAAAA GTTTTGGAGT ATTTTTGGAA TTTTTCAATC
      TAGTACGAGT AGCACTTTTT CAAAACCTCA TAAAAACCTT AAAAAGTTAG

      unc-54 3'  UTR
      ==================================================
2301  AAGTGAAAGT TTATGAAATT AATTTTCCTG CTTTTGCTTT TTGGGGGTTT
      TTCACTTTCA AATACTTTAA TTAAAAGGAC GAAAACGAAA AACCCCCAAA

      unc-54 3'  UTR
      ==================================================
2351  CCCCTATTGT TTGTCAAGAG TTTCGAGGAC GGCGTTTTTC TTGCTAAAAT
      GGGGATAACA AACAGTTCTC AAAGCTCCTG CCGCAAAAAG AACGATTTTA

      unc-54 3'  UTR
      ==================================================
2401  CACAAGTATT GATGAGCACG ATGCAAGAAA GATCGGAAGA AGGTTTGGGT
      GTGTTCATAA CTACTCGTGC TACGTTCTTT CTAGCCTTCT TCCAAACCCA

      unc-54 3'  UTR
      ==================================================
2451  TTGAGGCTCA GTGGAAGGTG AGTAGAAGTT GATAATTTGA AAGTGGAGTA
      AACTCCGAGT CACCTTCCAC TCATCTTCAA CTATTAAACT TTCACCTCAT

      unc-54 3'  UTR
      ==================================================
2501  GTGTCTATGG GGTTTTTGCC TTAAATGACA GAATACATTC CCAATATACC
      CACAGATACC CCAAAAACGG AATTTACTGT CTTATGTAAG GGTTATATGG

      unc-54 3'  UTR          MSC II
      ==============    ====================
2551  AAACATAACT GTTTCCTACT AGTCGGCCGT ACGGGCCCTT TCGTCTCGCG
      TTTGTATTGA CAAAGGATGA TCAGCCGGCA TGCCCGGGAA AGCAGAGCGC

2601  CGTTTCGGTG ATGACGGTGA AAACCTCTGA CACATGCAGC TCCCGGAGAC
      GCAAAGCCAC TACTGCCACT TTTGGAGACT GTGTACGTCG AGGGCCTCTG

2651  GGTCACAGCT TGTCTGTAAG CGGATGCCGG GAGCAGACAA GCCCGTCAGG
      CCAGTGTCGA ACAGACATTC GCCTACGGCC CTCGTCTGTT CGGGCAGTCC

2701  GCGCGTCAGC GGGTGTTGGC GGGTGTCGGG GCTGGCTTAA CTATGCGGCA
```

Fig. 16 continued

```
     CGCGCAGTCG CCCACAACCG CCCACAGCCC CGACCGAATT GATACGCCGT

2751 TCAGAGCAGA TTGTACTGAG AGTGCACCAT ATGCGGTGTG AAATACCGCA
     AGTCTCGTCT AACATGACTC TCACGTGGTA TACGCCACAC TTTATGGCGT

2801 CAGATGCGTA AGGAGAAAAT ACCGCATCAG GCGGCCTTAA GGGCCTCGTG
     GTCTACGCAT TCCTCTTTTA TGGCGTAGTC CGCCGGAATT CCCGGAGCAC

2851 ATACGCCTAT TTTTATAGGT TAATGTCATG ATAATAATGG TTTCTTAGAC
     TATGCGGATA AAAATATCCA ATTACAGTAC TATTATTACC AAAGAATCTG

2901 GTCAGGTGGC ACTTTTCGGG GAAATGTGCG CGGAACCCCT ATTTGTTTAT
     CAGTCCACCG TGAAAAGCCC CTTTACACGC GCCTTGGGGA TAAACAAATA

2951 TTTCTAAAT ACATTCAAAT ATGTATCCGC TCATGAGACA ATAACCCTGA
     AAAAGATTTA TGTAAGTTTA TACATAGGCG AGTACTCTGT TATTGGGACT

                                                      amp
                                            =================
3001 TAAATGCTTC AATAATATTG AAAAAGGAAG AGTATGAGTA TTCAACATTT
     ATTTACGAAG TTATTATAAC TTTTTCCTTC TCATACTCAT AAGTTGTAAA

                                                      amp
     ========================================================
3051 CCGTGTCGCC CTTATTCCCT TTTTTGCGGC ATTTTGCCTT CCTGTTTTTG
     GGCACAGCGG GAATAAGGGA AAAAACGCCG TAAAACGGAA GGACAAAAAC

                                                      amp
     ========================================================
3101 CTCACCCAGA AACGCTGGTG AAAGTAAAAG ATGCTGAAGA TCAGTTGGGT
     GAGTGGGTCT TTGCGACCAC TTTCATTTTC TACGACTTCT AGTCAACCCA

                                                      amp
     ========================================================
3151 GCACGAGTGG GTTACATCGA ACTGGATCTC AACAGCGGTA AGATCCTTGA
     CGTGCTCACC CAATGTAGCT TGACCTAGAG TTGTCGCCAT TCTAGGAACT

                                                      amp
     ========================================================
3201 GAGTTTTCGC CCCGAAGAAC GTTTTCCAAT GATGAGCACT TTTAAAGTTC
     CTCAAAAGCG GGGCTTCTTG CAAAAGGTTA CTACTCGTGA AAATTTCAAG

                                                      amp
     ========================================================
3251 TGCTATGTGG CGCGGTATTA TCCCGTATTG ACGCCGGGCA AGAGCAACTC
     ACGATACACC GCGCCATAAT AGGGCATAAC TGCGGCCCGT TCTCGTTGAG

                                                      amp
     ========================================================
3301 GGTCGCCGCA TACACTATTC TCAGAATGAC TTGGTTGAGT ACTCACCAGT
     CCAGCGGCGT ATGTGATAAG AGTCTTACTG AACCAACTCA TGAGTGGTCA

                                                      amp
     ========================================================
```

Fig. 16 continued

```
3351  CACAGAAAAG CATCTTACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG
      GTGTCTTTTC GTAGAATGCC TACCGTACTG TCATTCTCTT AATACGTCAC

                                                      amp
      ==================================================
3401  CTGCCATAAC CATGAGTGAT AACACTGCGG CCAACTTACT TCTGACAACG
      GACGGTATTG GTACTCACTA TTGTGACGCC GGTTGAATGA AGACTGTTGC

                   amp
      ==================================================
3451  ATCGGAGGAC CGAAGGAGCT AACCGCTTTT TTGCACAACA TGGGGGATCA
      TAGCCTCCTG GCTTCCTCGA TTGGCGAAAA AACGTGTTGT ACCCCCTAGT

      amp
      ==================================================
3501  TGTAACTCGC CTTGATCGTT GGGAACCGGA GCTGAATGAA GCCATACCAA
      ACATTGAGCG GAACTAGCAA CCCTTGGCCT CGACTTACTT CGGTATGGTT

      amp
      ==================================================
3551  ACGACGAGCG TGACACCACG ATGCCTGTAG CAATGGCAAC AACGTTGCGC
      TGCTGCTCGC ACTGTGGTGC TACGGACATC GTTACCGTTG TTGCAACGCG

      amp
      ==================================================
3601  AAACTATTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC AACAATTAAT
      TTTGATAATT GACCGCTTGA TGAATGAGAT CGAAGGGCCG TTGTTAATTA

      amp
      ==================================================
3651  AGACTGGATG GAGGCGGATA AAGTTGCAGG ACCACTTCTG CGCTCGGCCC
      TCTGACCTAC CTCCGCCTAT TTCAACGTCC TGGTGAAGAC GCGAGCCGGG

      amp
      ==================================================
3701  TTCCGGCTGG CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG
      AAGGCCGACC GACCAAATAA CGACTATTTA GACCTCGGCC ACTCGCACCC

      amp
      ==================================================
3751  TCTCGCGGTA TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGTAT
      AGAGCGCCAT AGTAACGTCG TGACCCCGGT CTACCATTCG GGAGGGCATA

      amp
      ==================================================
3801  CGTAGTTATC TACACGACGG GGAGTCAGGC AACTATGGAT GAACGAAATA
      GCATCAATAG ATGTGCTGCC CCTCAGTCCG TTGATACCTA CTTGCTTTAT

      amp
      ==========================================
3851  GACAGATCGC TGAGATAGGT GCCTCACTGA TTAAGCATTG GTAACTGTCA
      CTGTCTAGCG ACTCTATCCA CGGAGTGACT AATTCGTAAC CATTGACAGT

3901  GACCAAGTTT ACTCATATAT ACTTTAGATT GATTTAAAAC TTCATTTTTA
```

Fig. 16 continued

CTGGTTCAAA TGAGTATATA TGAAATCTAA CTAAATTTTG AAGTAAAAAT

3951 ATTTAAAAGG ATCTAGGTGA AGATCCTTTT TGATAATCTC ATGACCAAAA
TAAATTTTCC TAGATCCACT TCTAGGAAAA ACTATTAGAG TACTGGTTTT

4001 TCCCTTAACG TGAGTTTTCG TTCCACTGAG CGTCAGACCC CGTAGAAAAG
AGGGAATTGC ACTCAAAAGC AAGGTGACTC GCAGTCTGGG GCATCTTTTC

4051 ATCAAAGGAT CTTCTTGAGA TCCTTTTTTT CTGCGCGTAA TCTGCTGCTT
TAGTTTCCTA GAAGAACTCT AGGAAAAAAA GACGCGCATT AGACGACGAA

4101 GCAAACAAAA AAACCACCGC TACCAGCGGT GGTTTGTTTG CCGGATCAAG
CGTTTGTTTT TTTGGTGGCG ATGGTCGCCA CCAAACAAAC GGCCTAGTTC

4151 AGCTACCAAC TCTTTTTCCG AAGGTAACTG GCTTCAGCAG AGCGCAGATA
TCGATGGTTG AGAAAAAGGC TTCCATTGAC CGAAGTCGTC TCGCGTCTAT

4201 CCAAATACTG TCCTTCTAGT GTAGCCGTAG TTAGGCCACC ACTTCAAGAA
GGTTTATGAC AGGAAGATCA CATCGGCATC AATCCGGTGG TGAAGTTCTT

4251 CTCTGTAGCA CCGCCTACAT ACCTCGCTCT GCTAATCCTG TTACCAGTGG
GAGACATCGT GGCGGATGTA TGGAGCGAGA CGATTAGGAC AATGGTCACC

4301 CTGCTGCCAG TGGCGATAAG TCGTGTCTTA CCGGGTTGGA CTCAAGACGA
GACGACGGTC ACCGCTATTC AGCACAGAAT GGCCCAACCT GAGTTCTGCT

4351 TAGTTACCGG ATAAGGCGCA GCGGTCGGGC TGAACGGGGG GTTCGTGCAC
ATCAATGGCC TATTCCGCGT CGCCAGCCCG ACTTGCCCCC CAAGCACGTG

4401 ACAGCCCAGC TTGGAGCGAA CGACCTACAC CGAACTGAGA TACCTACAGC
TGTCGGGTCG AACCTCGCTT GCTGGATGTG GCTTGACTCT ATGGATGTCG

4451 GTGAGCATTG AGAAAGCGCC ACGCTTCCCG AAGGGAGAAA GGCGGACAGG
CACTCGTAAC TCTTTCGCGG TGCGAAGGGC TTCCCTCTTT CCGCCTGTCC

4501 TATCCGGTAA GCGGCAGGGT CGGAACAGGA GAGCGCACGA GGGAGCTTCC
ATAGGCCATT CGCCGTCCCA GCCTTGTCCT CTCGCGTGCT CCCTCGAAGG

4551 AGGGGGAAAC GCCTGGTATC TTTATAGTCC TGTCGGGTTT CGCCACCTCT
TCCCCCTTTG CGGACCATAG AAATATCAGG ACAGCCCAAA GCGGTGGAGA

4601 GACTTGAGCG TCGATTTTTG TGATGCTCGT CAGGGGGGCG GAGCCTATGG
CTGAACTCGC AGCTAAAAAC ACTACGAGCA GTCCCCCCGC CTCGGATACC

4651 AAAAACGCCA GCAACGCGGC CTTTTTACGG TTCCTGGCCT TTTGCTGGCC
TTTTTGCGGT CGTTGCGCCG GAAAAATGCC AAGGACCGGA AAACGACCGG

4701 TTTTGCTCAC ATGTTCTTTC CTGCGTTATC CCCTGATTCT GTGGATAACC
AAAACGAGTG TACAAGAAAG GACGCAATAG GGGACTAAGA CACCTATTGG

4751 GTATTACCGC CTTTGAGTGA GCTGATACCG CTCGCCGCAG CCGAACGACC
CATAATGGCG GAAACTCACT CGACTATGGC GAGCGGCGTC GGCTTGCTGG

4801 GAGCGCAGCG AGTCAGTGAG CGAGGAAGCG GAAGAGCGCC CAATACGCAA

Fig. 16 continued

```
     CTCGCGTCGC TCAGTCACTC GCTCCTTCGC CTTCTCGCGG GTTATGCGTT

4851 ACCGCCTCTC CCCGCGCGTT GGCCGATTCA TTAATGCAGC TGGCACGACA
     TGGCGGAGAG GGGCGCGCAA CCGGCTAAGT AATTACGTCG ACCGTGCTGT

4901 GGTTTCCCGA CTGGAAAGCG GGCAGTGAGC GCAACGCAAT TAATGTGAGT
     CCAAAGGGCT GACCTTTCGC CCGTCACTCG CGTTGCGTTA ATTACACTCA

4951 TAGCTCACTC ATTAGGCACC CCAGGCTTTA CACTTTATGC TTCCGGCTCG
     ATCGAGTGAG TAATCCGTGG GGTCCGAAAT GTGAAATACG AAGGCCGAGC

5001 TATGTTGTGT GGAATTGTGA GCGGATAACA ATTTCACACA GGAAACAGCT
     ATACAACACA CCTTAACACT CGCCTATTGT TAAAGTGTGT CCTTTGTCGA

5051 ATGACCATGA TTACGCCAAG CTGTAAGTTT AAACATGATC TTACTAACTA
     TACTGGTACT AATGCGGTTC GACATTCAAA TTTGTACTAG AATGATTGAT

5101 ACTATTCTCA TTTAAATTTT CAGAGCTTAA AAATGGCTGA AATCACTCAC
     TGATAAGAGT AAATTTAAAA GTCTCGAATT TTTACCGACT TTAGTGAGTG

5151 AACGATGGAT ACGCTAACAA CTTGGAAATG AAATAAGCTT GCATGCCTGC
     TTGCTACCTA TGCGATTGTT GAACCTTTAC TTTATTCGAA CGTACGGACG

                                                vit-2  promoter
                                     ==========================
      StuI
     ~~~~~~
5201 AGGCCTTGGT CGACTCTAGA GGATCAAACT GTATTACTTG AAACAATTTA
     TCCGGAACCA GCTGAGATCT CCTAGTTTGA CATAATGAAC TTTGTTAAAT

                                                vit-2  promoter
     ==========================================================
5251 GTTATATGTT TAGAACCCCT CATTCAAAAT TAATAGACAG GGCTCTCACC
     CAATATACAA ATCTTGGGGA GTAAGTTTTA ATTATCTGTC CCGAGAGTGG

                                                vit-2  promoter
     ==========================================================
5301 GAATGTTGCA ATTTGTTTCT GATAAGGGTC ACAAAGCGGA GCGAATGCTT
     CTTACAACGT TAAACAAAGA CTATTCCCAG TGTTTCGCCT CGCTTACGAA

     vit-2 promoter
     ==========================================================
5351 GAATGTGTCC ATCAATGAGC TTATCAATGC GCTAAAACGC TATAACTTCC
     CTTACACAGG TAGTTACTCG AATAGTTACG CGATTTTGCG ATATTGAAGG

     vit-2 promoter
     ==========================================================
5401 ATATGAAGTC AATCGAACAT ATGTCAATCT TTAGCCGTAT ATAAAGGTGC
     TATACTTCAG TTAGCTTGTA TACAGTTAGA AATCGGCATA TATTTCCACG

     vit-2 promoter            exon 1  (in frame  - partial)
     ==========================================================
5451 ACTGAAAACA GTCCAATCAC GGTTCAGCCA TGAGGTCGAT CCCCGGCCGG
     TGACTTTTGT CAGGTTAGTG CCAAGTCGGT ACTCCAGCTA GGGGCCGGCC
```

Fig. 16 continued

```
     exon 1 (in  frame - partial)          synth.  intron
     ======================================================
5501 GATTGGCCAA AGGACCCAAA GGTATGTTTC GAATGATACT AACATAACAT
     CTAACCGGTT TCCTGGGTTT CCATACAAAG CTTACTATGA TTGTATTGTA

     synth. intron
     ==============
5551 AGAACATTTT CAGGAGGACC CTTGGAGGGT ACCGGGGATT GGCCAAAGGA
     TCTTGTAAAA GTCCTCCTGG GAACCTCCCA TGGCCCCTAA CCGGTTTCCT

5601 CCCAAAGGTA TGTTTCGAAT GATACTAACA TAACATAGAA CATTTTCAGG
     GGGTTTCCAT ACAAAGCTTA CTATGATTGT ATTGTATCTT GTAAAAGTCC

                                SacI
                               ~~~~~~
5651 AGGACCCTTG CTTGGAGGGT ACCGAGCTCA GAAAAA
     TCCTGGGAAC GAACCTCCCA TGGCTCGAGT CTTTTT
```

Fig. 17

III. Predicted DNA sequence pGQ2

```
                              NLS                    luc+
     ==================================================
  1  ATGACTGCTC CAAAGAAGAA GCGTAAGGTA CCGGTAGAAA AAATGGAAGA
     TACTGACGAG GTTTCTTCTT CGCATTCCAT GGCCATCTTT TTTACCTTCT

                                                     luc+
     ==================================================
 51  CGCCAAAAAC ATAAAGAAAG GCCCGGCGCC ATTCTATCCG CTGGAAGATG
     GCGGTTTTTG TATTTCTTTC CGGGCCGCGG TAAGATAGGC GACCTTCTAC

                                                     luc+
     ==================================================
101  GAACCGCTGG AGAGCAACTG CATAAGGCTA TGAAGAGATA CGCCCTGGTT
     CTTGGCGACC TCTCGTTGAC GTATTCCGAT ACTTCTCTAT GCGGGACCAA

                                                     luc+
     ==================================================
151  CCTGGAACAA TTGCTTTTAC AGATGCACAT ATCGAGGTGG ACATCACTTA
     GGACCTTGTT AACGAAAATG TCTACGTGTA TAGCTCCACC TGTAGTGAAT

                                                     luc+
     ==================================================
201  CGCTGAGTAC TTCGAAATGT CCGTTCGGTT GGCAGAAGCT ATGAAACGAT
     GCGACTCATG AAGCTTTACA GGCAAGCCAA CCGTCTTCGA TACTTTGCTA

                                                     luc+
     ==================================================
251  ATGGGCTGAA TACAAATCAC AGAATCGTCG TATGCAGTGA AAACTCTCTT
     TACCCGACTT ATGTTTAGTG TCTTAGCAGC ATACGTCACT TTTGAGAGAA

                                                     luc+
     ==================================================
301  CAATTCTTTA TGCCGGTGTT GGGCGCGTTA TTTATCGGAG TTGCAGTTGC
     GTTAAGAAAT ACGGCCACAA CCCGCGCAAT AAATAGCCTC AACGTCAACG

                                                     luc+
     ==================================================
351  GCCCGCGAAC GACATTTATA ATGAACGTGA ATTGCTCAAC AGTATGGGCA
     CGGGCGCTTG CTGTAAATAT TACTTGCACT TAACGAGTTG TCATACCCGT

                                                     luc+
     ==================================================
401  TTTCGCAGCC TACCGTGGTG TTCGTTTCCA AAAAGGGGTT GCAAAAAATT
     AAAGCGTCGG ATGGCACCAC AAGCAAAGGT TTTTCCCCAA CGTTTTTTAA

                                                     luc+
     ==================================================
451  TTGAACGTGC AAAAAAAGCT CCCAATCATC CAAAAAATTA TTATCATGGA
     AACTTGCACG TTTTTTTCGA GGGTTAGTAG GTTTTTTAAT AATAGTACCT
```

Fig. 17 continued

```
                                                            luc+
         ====================================================
501      TTCTAAAACG GATTACCAGG GATTTCAGTC GATGTACACG TTCGTCACAT
         AAGATTTTGC CTAATGGTCC CTAAAGTCAG CTACATGTGC AAGCAGTGTA

                                                            luc+
         ====================================================
551      CTCATCTACC TCCCGGTTTT AATGAATACG ATTTTGTGCC AGAGTCCTTC
         GAGTAGATGG AGGGCCAAAA TTACTTATGC TAAAACACGG TCTCAGGAAG

                                                            luc+
         ====================================================
601      GATAGGGACA AGACAATTGC ACTGATCATG AACTCCTCTG GATCTACTGG
         CTATCCCTGT TCTGTTAACG TGACTAGTAC TTGAGGAGAC CTAGATGACC

                                                            luc+
         ====================================================
651      TCTGCCTAAA GGTGTCGCTC TGCCTCATAG AACTGCCTGC GTGAGATTCT
         AGACGGATTT CCACAGCGAG ACGGAGTATC TTGACGGACG CACTCTAAGA

                                                            luc+
         ====================================================
701      CGCATGCCAG AGATCCTATT TTTGGCAATC AAATCATTCC GGATACTGCG
         GCGTACGGTC TCTAGGATAA AAACCGTTAG TTTAGTAAGG CCTATGACGC

                                                            luc+
         ====================================================
751      ATTTTAAGTG TTGTTCCATT CCATCACGGT TTTGGAATGT TTACTACACT
         TAAAATTCAC AACAAGGTAA GGTAGTGCCA AAACCTTACA AATGATGTGA

                                                            luc+
         ====================================================
801      CGGATATTTG ATATGTGGAT TTCGAGTCGT CTTAATGTAT AGATTTGAAG
         GCCTATAAAC TATACACCTA AAGCTCAGCA GAATTACATA TCTAAACTTC

                              luc+
         ====================================================
851      AAGAGCTGTT TCTGAGGAGC CTTCAGGATT ACAAGATTCA AAGTGCGCTG
         TTCTCGACAA AGACTCCTCG GAAGTCCTAA TGTTCTAAGT TTCACGCGAC

         luc+
         ====================================================
901      CTGGTGCCAA CCCTATTCTC CTTCTTCGCC AAAAGCACTC TGATTGACAA
         GACCACGGTT GGGATAAGAG GAAGAAGCGG TTTTCGTGAG ACTAACTGTT

         luc+
         ====================================================
951      ATACGATTTA TCTAATTTAC ACGAAATTGC TTCTGGTGGC GCTCCCCTCT
         TATGCTAAAT AGATTAAATG TGCTTTAACG AAGACCACCG CGAGGGGAGA

         luc+
         ====================================================
1001     CTAAGGAAGT CGGGGAAGCG GTTGCCAAGA GGTTCCATCT GCCAGGTATC
         GATTCCTTCA GCCCCTTCGC CAACGGTTCT CCAAGGTAGA CGGTCCATAG
```

Fig. 17 continued

```
     luc+
     ==================================================
1051 AGGCAAGGAT ATGGGCTCAC TGAGACTACA TCAGCTATTC TGATTACACC
     TCCGTTCCTA TACCCGAGTG ACTCTGATGT AGTCGATAAG ACTAATGTGG

     luc+
     ==================================================
1101 CGAGGGGGAT GATAAACCGG GCGCGGTCGG TAAAGTTGTT CCATTTTTTG
     GCTCCCCCTA CTATTTGGCC CGCGCCAGCC ATTTCAACAA GGTAAAAAAC

     luc+
     ==================================================
1151 AAGCGAAGGT TGTGGATCTG GATACCGGGA AAACGCTGGG CGTTAATCAA
     TTCGCTTCCA ACACCTAGAC CTATGGCCCT TTTGCGACCC GCAATTAGTT

     luc+
     ==================================================
1201 AGAGGCGAAC TGTGTGTGAG AGGTCCTATG ATTATGTCCG GTTATGTAAA
     TCTCCGCTTG ACACACACTC TCCAGGATAC TAATACAGGC CAATACATTT

     luc+
     ==================================================
1251 CAATCCGGAA GCGACCAACG CCTTGATTGA CAAGGATGGA TGGCTACATT
     GTTAGGCCTT CGCTGGTTGC GGAACTAACT GTTCCTACCT ACCGATGTAA

     luc+
     ==================================================
1301 CTGGAGACAT AGCTTACTGG GACGAAGACG AACACTTCTT CATCGTTGAC
     GACCTCTGTA TCGAATGACC CTGCTTCTGC TTGTGAAGAA GTAGCAACTG

     luc+
     ==================================================
1351 CGCCTGAAGT CTCTGATTAA GTACAAAGGC TATCAGGTGG CTCCCGCTGA
     GCGGACTTCA GAGACTAATT CATGTTTCCG ATAGTCCACC GAGGGCGACT

     luc+
     ==================================================
1401 ATTGGAATCC ATCTTGCTCC AACACCCCAA CATCTTCGAC GCAGGTGTCG
     TAACCTTAGG TAGAACGAGG TTGTGGGGTT GTAGAAGCTG CGTCCACAGC

     luc+
     ==================================================
1451 CAGGTCTTCC CGACGATGAC GCCGGTGAAC TTCCCGCCGC CGTTGTTGTT
     GTCCAGAAGG GCTGCTACTG CGGCCACTTG AAGGGCGGCG GCAACAACAA

     luc+
     ==================================================
1501 TTGGAGCACG GAAAGACGAT GACGGAAAAA GAGATCGTGG ATTACGTCGC
     AACCTCGTGC CTTTCTGCTA CTGCCTTTTT CTCTAGCACC TAATGCAGCG

     luc+
     ==================================================
1551 CAGTCAAGTA ACAACCGCGA AAAAGTTGCG CGGAGGAGTT GTGTTTGTGG
```

Fig. 17 continued

```
      GTCAGTTCAT TGTTGGCGCT TTTTCAACGC GCCTCCTCAA CACAAACACC

      luc+
      ==================================================
1601  ACGAAGTACC GAAAGGTCTT ACCGGAAAAC TCGACGCAAG AAAAATCAGA
      TGCTTCATGG CTTTCCAGAA TGGCCTTTTG AGCTGCGTTC TTTTTAGTCT

      luc+
      ============================================
1651  GAGATCCTCA TAAAGGCCAA GAAGGGCGGA AAGATCGCCG TGTAATTCTA
      CTCTAGGAGT ATTTCCGGTT CTTCCCGCCT TTCTAGCGGC ACATTAAGAT

                                           unc-54  3' UTR
                ========================================
1701  GGAATTCCAA CTGAGCGCCG GTCGCTACCA TTACCAACTT GTCTGGTGTC
      CCTTAAGGTT GACTCGCGGC CAGCGATGGT AATGGTTGAA CAGACCACAG

                                           unc-54  3' UTR
      ==================================================
1751  AAAAATAATA GGGGCCGCTG TCATCAGAGT AAGTTTAAAC TGAGTTCTAC
      TTTTTATTAT CCCCGGCGAC AGTAGTCTCA TTCAAATTTG ACTCAAGATG

                                           unc-54  3' UTR
      ==================================================
1801  TAACTAACGA GTAATATTTA AATTTTCAGC ATCTCGCGCC CGTGCCTCTG
      ATTGATTGCT CATTATAAAT TTAAAAGTCG TAGAGCGCGG GCACGGAGAC

                                           unc-54  3' UTR
      ==================================================
1851  ACTTCTAAGT CCAATTACTC TTCAACATCC CTACATGCTC TTTCTCCCTG
      TGAAGATTCA GGTTAATGAG AAGTTGTAGG GATGTACGAG AAAGAGGGAC

                                           unc-54  3' UTR
      ==================================================
1901  TGCTCCCACC CCCTATTTTT GTTATTATCA AAAAAACTTC TTCTTAATTT
      ACGAGGGTGG GGGATAAAAA CAATAATAGT TTTTTTGAAG AAGAATTAAA

                                           unc-54  3' UTR
      ==================================================
1951  CTTTGTTTTT TAGCTTCTTT TAAGTCACCT CTAACAATGA AATTGTGTAG
      GAAACAAAAA ATCGAAGAAA ATTCAGTGGA GATTGTTACT TTAACACATC

                                           unc-54  3' UTR
      ==================================================
2001  ATTCAAAAAT AGAATTAATT CGTAATAAAA AGTCGAAAAA AATTGTGCTC
      TAAGTTTTTA TCTTAATTAA GCATTATTTT TCAGCTTTTT TTAACACGAG

                                           unc-54  3' UTR
      ==================================================
2051  CCTCCCCCCA TTAATAATAA TTCTATCCCA AAATCTACAC AATGTTCTGT
      GGAGGGGGGT AATTATTATT AAGATAGGGT TTTAGATGTG TTACAAGACA

                                unc-54  3' UTR
      ==================================================
```

Fig. 17 continued

```
2101  GTACACTTCT TATGTTTTTT TTACTTCTGA TAAATTTTTT TTGAAACATC
      CATGTGAAGA ATACAAAAAA AATGAAGACT ATTTAAAAAA AACTTTGTAG

      unc-54 3'  UTR
      ==================================================
2151  ATAGAAAAAA CCGCACACAA AATACCTTAT CATATGTTAC GTTTCAGTTT
      TATCTTTTTT GGCGTGTGTT TTATGGAATA GTATACAATG CAAAGTCAAA

      unc-54 3'  UTR
      ==================================================
2201  ATGACCGCAA TTTTTATTTC TTCGCACGTC TGGGCCTCTC ATGACGTCAA
      TACTGGCGTT AAAAATAAAG AAGCGTGCAG ACCCGGAGAG TACTGCAGTT

      unc-54 3'  UTR
      ==================================================
2251  ATCATGCTCA TCGTGAAAAA GTTTTGGAGT ATTTTTGGAA TTTTTCAATC
      TAGTACGAGT AGCACTTTTT CAAAACCTCA TAAAAACCTT AAAAAGTTAG

      unc-54 3'  UTR
      ==================================================
2301  AAGTGAAAGT TTATGAAATT AATTTTCCTG CTTTTGCTTT TTGGGGGTTT
      TTCACTTTCA AATACTTTAA TTAAAAGGAC GAAAACGAAA AACCCCCAAA

      unc-54 3'  UTR
      ==================================================
2351  CCCCTATTGT TTGTCAAGAG TTTCGAGGAC GGCGTTTTTC TTGCTAAAAT
      GGGGATAACA AACAGTTCTC AAAGCTCCTG CCGCAAAAAG AACGATTTTA

      unc-54 3'  UTR
      ==================================================
2401  CACAAGTATT GATGAGCACG ATGCAAGAAA GATCGGAAGA AGGTTTGGGT
      GTGTTCATAA CTACTCGTGC TACGTTCTTT CTAGCCTTCT TCCAAACCCA

      unc-54 3'  UTR
      ==================================================
2451  TTGAGGCTCA GTGGAAGGTG AGTAGAAGTT GATAATTTGA AAGTGGAGTA
      AACTCCGAGT CACCTTCCAC TCATCTTCAA CTATTAAACT TTCACCTCAT

      unc-54 3'  UTR
      ==================================================
2501  GTGTCTATGG GGTTTTTGCC TTAAATGACA GAATACATTC CCAATATACC
      CACAGATACC CCAAAAACGG AATTTACTGT CTTATGTAAG GGTTATATGG

      unc-54 3'  UTR          MSC II
      ===============    =====================
2551  AAACATAACT GTTTCCTACT AGTCGGCCGT ACGGGCCCTT TCGTCTCGCG
      TTTGTATTGA CAAAGGATGA TCAGCCGGCA TGCCCGGGAA AGCAGAGCGC

2601  CGTTTCGGTG ATGACGGTGA AAACCTCTGA CACATGCAGC TCCCGGAGAC
      GCAAAGCCAC TACTGCCACT TTTGGAGACT GTGTACGTCG AGGGCCTCTG

2651  GGTCACAGCT TGTCTGTAAG CGGATGCCGG GAGCAGACAA GCCCGTCAGG
      CCAGTGTCGA ACAGACATTC GCCTACGGCC CTCGTCTGTT CGGGCAGTCC
```

Fig. 17 continued

```
2701  GCGCGTCAGC GGGTGTTGGC GGGTGTCGGG GCTGGCTTAA CTATGCGGCA
      CGCGCAGTCG CCCACAACCG CCCACAGCCC CGACCGAATT GATACGCCGT

2751  TCAGAGCAGA TTGTACTGAG AGTGCACCAT ATGCGGTGTG AAATACCGCA
      AGTCTCGTCT AACATGACTC TCACGTGGTA TACGCCACAC TTTATGGCGT

2801  CAGATGCGTA AGGAGAAAAT ACCGCATCAG GCGGCCTTAA GGGCCTCGTG
      GTCTACGCAT TCCTCTTTTA TGGCGTAGTC CGCCGGAATT CCCGGAGCAC

2851  ATACGCCTAT TTTTATAGGT TAATGTCATG ATAATAATGG TTTCTTAGAC
      TATGCGGATA AAAATATCCA ATTACAGTAC TATTATTACC AAAGAATCTG

2901  GTCAGGTGGC ACTTTTCGGG GAAATGTGCG CGGAACCCCT ATTTGTTTAT
      CAGTCCACCG TGAAAAGCCC CTTTACACGC GCCTTGGGGA TAAACAAATA

2951  TTTTCTAAAT ACATTCAAAT ATGTATCCGC TCATGAGACA ATAACCCTGA
      AAAAGATTTA TGTAAGTTTA TACATAGGCG AGTACTCTGT TATTGGGACT

                                                        amp
                                            ===============
3001  TAAATGCTTC AATAATATTG AAAAAGGAAG AGTATGAGTA TTCAACATTT
      ATTTACGAAG TTATTATAAC TTTTTCCTTC TCATACTCAT AAGTTGTAAA

                                                        amp
      =====================================================
3051  CCGTGTCGCC CTTATTCCCT TTTTTGCGGC ATTTTGCCTT CCTGTTTTTG
      GGCACAGCGG GAATAAGGGA AAAAACGCCG TAAAACGGAA GGACAAAAAC

                                                        amp
      =====================================================
3101  CTCACCCAGA AACGCTGGTG AAAGTAAAAG ATGCTGAAGA TCAGTTGGGT
      GAGTGGGTCT TTGCGACCAC TTTCATTTTC TACGACTTCT AGTCAACCCA

                                                        amp
      =====================================================
3151  GCACGAGTGG GTTACATCGA ACTGGATCTC AACAGCGGTA AGATCCTTGA
      CGTGCTCACC CAATGTAGCT TGACCTAGAG TTGTCGCCAT TCTAGGAACT

                                                        amp
      =====================================================
3201  GAGTTTTCGC CCCGAAGAAC GTTTTCCAAT GATGAGCACT TTTAAAGTTC
      CTCAAAAGCG GGGCTTCTTG CAAAAGGTTA CTACTCGTGA AAATTTCAAG

                                                        amp
      =====================================================
3251  TGCTATGTGG CGCGGTATTA TCCCGTATTG ACGCCGGGCA AGAGCAACTC
      ACGATACACC GCGCCATAAT AGGGCATAAC TGCGGCCCGT TCTCGTTGAG

                                                        amp
      =====================================================
3301  GGTCGCCGCA TACACTATTC TCAGAATGAC TTGGTTGAGT ACTCACCAGT
      CCAGCGGCGT ATGTGATAAG AGTCTTACTG AACCAACTCA TGAGTGGTCA

                                                        amp
```

Fig. 17 continued

```
     ==================================================
3351 CACAGAAAAG CATCTTACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG
     GTGTCTTTTC GTAGAATGCC TACCGTACTG TCATTCTCTT AATACGTCAC

                                                    amp
     ==================================================
3401 CTGCCATAAC CATGAGTGAT AACACTGCGG CCAACTTACT TCTGACAACG
     GACGGTATTG GTACTCACTA TTGTGACGCC GGTTGAATGA AGACTGTTGC

                 amp
     ==================================================
3451 ATCGGAGGAC CGAAGGAGCT AACCGCTTTT TTGCACAACA TGGGGGATCA
     TAGCCTCCTG GCTTCCTCGA TTGGCGAAAA AACGTGTTGT ACCCCCTAGT

     amp
     ==================================================
3501 TGTAACTCGC CTTGATCGTT GGGAACCGGA GCTGAATGAA GCCATACCAA
     ACATTGAGCG GAACTAGCAA CCCTTGGCCT CGACTTACTT CGGTATGGTT

     amp
     ==================================================
3551 ACGACGAGCG TGACACCACG ATGCCTGTAG CAATGGCAAC AACGTTGCGC
     TGCTGCTCGC ACTGTGGTGC TACGGACATC GTTACCGTTG TTGCAACGCG

     amp
     ==================================================
3601 AAACTATTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC AACAATTAAT
     TTTGATAATT GACCGCTTGA TGAATGAGAT CGAAGGGCCG TTGTTAATTA

     amp
     ==================================================
3651 AGACTGGATG GAGGCGGATA AAGTTGCAGG ACCACTTCTG CGCTCGGCCC
     TCTGACCTAC CTCCGCCTAT TTCAACGTCC TGGTGAAGAC GCGAGCCGGG

     amp
     ==================================================
3701 TTCCGGCTGG CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG
     AAGGCCGACC GACCAAATAA CGACTATTTA GACCTCGGCC ACTCGCACCC

     amp
     ==================================================
3751 TCTCGCGGTA TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGTAT
     AGAGCGCCAT AGTAACGTCG TGACCCCGGT CTACCATTCG GGAGGGCATA

     amp
     ==================================================
3801 CGTAGTTATC TACACGACGG GGAGTCAGGC AACTATGGAT GAACGAAATA
     GCATCAATAG ATGTGCTGCC CCTCAGTCCG TTGATACCTA CTTGCTTTAT

     amp
     ========================================
3851 GACAGATCGC TGAGATAGGT GCCTCACTGA TTAAGCATTG GTAACTGTCA
     CTGTCTAGCG ACTCTATCCA CGGAGTGACT AATTCGTAAC CATTGACAGT
```

Fig. 17 continued

```
3901  GACCAAGTTT ACTCATATAT ACTTTAGATT GATTTAAAAC TTCATTTTTA
      CTGGTTCAAA TGAGTATATA TGAAATCTAA CTAAATTTTG AAGTAAAAAT

3951  ATTTAAAAGG ATCTAGGTGA AGATCCTTTT TGATAATCTC ATGACCAAAA
      TAAATTTTCC TAGATCCACT TCTAGGAAAA ACTATTAGAG TACTGGTTTT

4001  TCCCTTAACG TGAGTTTTCG TTCCACTGAG CGTCAGACCC CGTAGAAAAG
      AGGGAATTGC ACTCAAAAGC AAGGTGACTC GCAGTCTGGG GCATCTTTTC

4051  ATCAAAGGAT CTTCTTGAGA TCCTTTTTTT CTGCGCGTAA TCTGCTGCTT
      TAGTTTCCTA GAAGAACTCT AGGAAAAAAA GACGCGCATT AGACGACGAA

4101  GCAAACAAAA AAACCACCGC TACCAGCGGT GGTTTGTTTG CCGGATCAAG
      CGTTTGTTTT TTTGGTGGCG ATGGTCGCCA CCAAACAAAC GGCCTAGTTC

4151  AGCTACCAAC TCTTTTTCCG AAGGTAACTG GCTTCAGCAG AGCGCAGATA
      TCGATGGTTG AGAAAAAGGC TTCCATTGAC CGAAGTCGTC TCGCGTCTAT

4201  CCAAATACTG TCCTTCTAGT GTAGCCGTAG TTAGGCCACC ACTTCAAGAA
      GGTTTATGAC AGGAAGATCA CATCGGCATC AATCCGGTGG TGAAGTTCTT

4251  CTCTGTAGCA CCGCCTACAT ACCTCGCTCT GCTAATCCTG TTACCAGTGG
      GAGACATCGT GGCGGATGTA TGGAGCGAGA CGATTAGGAC AATGGTCACC

4301  CTGCTGCCAG TGGCGATAAG TCGTGTCTTA CCGGGTTGGA CTCAAGACGA
      GACGACGGTC ACCGCTATTC AGCACAGAAT GGCCCAACCT GAGTTCTGCT

4351  TAGTTACCGG ATAAGGCGCA GCGGTCGGGC TGAACGGGGG GTTCGTGCAC
      ATCAATGGCC TATTCCGCGT CGCCAGCCCG ACTTGCCCCC CAAGCACGTG

4401  ACAGCCCAGC TTGGAGCGAA CGACCTACAC CGAACTGAGA TACCTACAGC
      TGTCGGGTCG AACCTCGCTT GCTGGATGTG GCTTGACTCT ATGGATGTCG

4451  GTGAGCATTG AGAAAGCGCC ACGCTTCCCG AAGGGAGAAA GGCGGACAGG
      CACTCGTAAC TCTTTCGCGG TGCGAAGGGC TTCCCTCTTT CCGCCTGTCC

4501  TATCCGGTAA GCGGCAGGGT CGGAACAGGA GAGCGCACGA GGGAGCTTCC
      ATAGGCCATT CGCCGTCCCA GCCTTGTCCT CTCGCGTGCT CCCTCGAAGG

4551  AGGGGGAAAC GCCTGGTATC TTTATAGTCC TGTCGGGTTT CGCCACCTCT
      TCCCCCTTTG CGGACCATAG AAATATCAGG ACAGCCCAAA GCGGTGGAGA

4601  GACTTGAGCG TCGATTTTTG TGATGCTCGT CAGGGGGGCG GAGCCTATGG
      CTGAACTCGC AGCTAAAAAC ACTACGAGCA GTCCCCCCGC CTCGGATACC

4651  AAAAACGCCA GCAACGCGGC CTTTTTACGG TTCCTGGCCT TTTGCTGGCC
      TTTTTGCGGT CGTTGCGCCG GAAAAATGCC AAGGACCGGA AAACGACCGG

4701  TTTTGCTCAC ATGTTCTTTC CTGCGTTATC CCCTGATTCT GTGGATAACC
      AAAACGAGTG TACAAGAAAG GACGCAATAG GGGACTAAGA CACCTATTGG

4751  GTATTACCGC CTTTGAGTGA GCTGATACCG CTCGCCGCAG CCGAACGACC
      CATAATGGCG GAAACTCACT CGACTATGGC GAGCGGCGTC GGCTTGCTGG
```

Fig. 17 continued

```
4801  GAGCGCAGCG AGTCAGTGAG CGAGGAAGCG GAAGAGCGCC CAATACGCAA
      CTCGCGTCGC TCAGTCACTC GCTCCTTCGC CTTCTCGCGG GTTATGCGTT

4851  ACCGCCTCTC CCCGCGCGTT GGCCGATTCA TTAATGCAGC TGGCACGACA
      TGGCGGAGAG GGGCGCGCAA CCGGCTAAGT AATTACGTCG ACCGTGCTGT

4901  GGTTTCCCGA CTGGAAAGCG GGCAGTGAGC GCAACGCAAT TAATGTGAGT
      CCAAAGGGCT GACCTTTCGC CCGTCACTCG CGTTGCGTTA ATTACACTCA

4951  TAGCTCACTC ATTAGGCACC CCAGGCTTTA CACTTTATGC TTCCGGCTCG
      ATCGAGTGAG TAATCCGTGG GGTCCGAAAT GTGAAATACG AAGGCCGAGC

5001  TATGTTGTGT GGAATTGTGA GCGGATAACA ATTTCACACA GGAAACAGCT
      ATACAACACA CCTTAACACT CGCCTATTGT TAAAGTGTGT CCTTTGTCGA

5051  ATGACCATGA TTACGCCAAG CTGTAAGTTT AAACATGATC TTACTAACTA
      TACTGGTACT AATGCGGTTC GACATTCAAA TTTGTACTAG AATGATTGAT

5101  ACTATTCTCA TTTAAATTTT CAGAGCTTAA AAATGGCTGA AATCACTCAC
      TGATAAGAGT AAATTTAAAA GTCTCGAATT TTTACCGACT TTAGTGAGTG

5151  AACGATGGAT ACGCTAACAA CTTGGAAATG AAATAAGCTT GCATGCCTGC
      TTGCTACCTA TGCGATTGTT GAACCTTTAC TTTATTCGAA CGTACGGACG

                                  ctl-1 promoter  + coding  region
         ===================================================
                               o-GQ3
         ========================================
       StuI
      ~~~~~~
5201  AGGCCTGAGA TATTTTGCGC GTCAAATATG TTTTGTGTCC CCGTAATATT
      TCCGGACTCT ATAAAACGCG CAGTTTATAC AAAACACAGG GGCATTATAA

                                  ctl-1 promoter  + coding  region
      ======================================================
5251  TTTTTAAATC AAATTTCACA TTTTAACCAT AAAAAACTCT TTCAAAAGTG
      AAAAATTTAG TTTAAAGTGT AAAATTGGTA TTTTTTGAGA AAGTTTTCAC

                                  ctl-1 promoter  + coding  region
      ======================================================
5301  TAATTTTCTA CGCAAAAATG CCGTTCGGAT GAAAAATTAC TTTTGAAAAA
      ATTAAAAGAT GCGTTTTTAC GGCAAGCCTA CTTTTTAATG AAAACTTTTT

                                  ctl-1 promoter  + coding  region
      ======================================================
5351  CAAACTCGAA ACTACGGTAC GCAAAAAAGT ACATCGGTGT TTGCACATAA
      GTTTGAGCTT TGATGCCATG CGTTTTTTCA TGTAGCCACA AACGTGTATT

                                  ctl-1 promoter  + coding  region
      ======================================================
5401  GTGAAAACAA TGTTGTTTTT TTGTAATTAA AATCGATTAA TTTTTTTTCC
      CACTTTTGTT ACAACAAAAA AACATTAATT TTAGCTAATT AAAAAAAAGG

                                  ctl-1 promoter  + coding  region
```

Fig. 17 continued

```
       ==================================================
5451   CGGAAAACAA AAACGTTTTC AGCGTGGATT TCTATTGTTT CTTGCGTAAA
       GCCTTTTGTT TTTGCAAAAG TCGCACCTAA AGATAACAAA GAACGCATTT

                                ctl-1 promoter  + coding  region
       ==================================================
5501   AAAAAATTAT TTACCAATTT TAAACGATAA TTTCCACGAA TTTTCGCCAT
       TTTTTTAATA AATGGTTAAA ATTTGCTATT AAAGGTGCTT AAAAGCGGTA

                                ctl-1 promoter  + coding  region
       ==================================================
5551   TAATCTCTCG ATTTTGTTGA TTCTTGACTC CGAGCAATCT CTCCGGTTTT
       ATTAGAGAGC TAAAACAACT AAGAACTGAG GCTCGTTAGA GAGGCCAAAA

                                ctl-1 promoter  + coding  region
       ==================================================
5601   CGCAAACGAT TATATTATTT ATTTGTTTTC CTTTTCAGTG CCGATTCTCG
       GCGTTTGCTA ATATAATAAA TAAACAAAAG GAAAAGTCAC GGCTAAGAGC

       ctl-1 promoter  + coding  region
       ==================================================
                                                Exon  1
                                  ========================
5651   GAAATTCAAC AGTAAATCTT CAAAATGCCA ATGCTTCCCC ACATGGTCAA
       CTTTAAGTTG TCATTTAGAA GTTTTACGGT TACGAAGGGG TGTACCAGTT

       ctl-1 promoter  + coding  region
       ==================================================
       Exon 1
       =====
5701   TCTAAGTGAG TTTCTTTGTT ACAAAATACA CGTGATGTCA GATTGTCTCA
       AGATTCACTC AAAGAAACAA TGTTTTATGT GCACTACAGT CTAACAGAGT

       ctl-1 promoter  + coding  region
       ==================================================
5751   TTTCGGTTTG ATCTACGTAG ATCTACAAAA AATGCGGGAA TTGAGCCGCA
       AAAGCCAAAC TAGATGCATC TAGATGTTTT TTACGCCCTT AACTCGGCGT

       ctl-1 promoter  + coding  region
       ==================================================
5801   GAGTTCTCAA CTGCTTTCGC ATGGTTAAGA ACGTGCGGAC GTCAAATTGT
       CTCAAGAGTT GACGAAAGCG TACCAATTCT TGCACGCCTG CAGTTTAACA

       ctl-1 promoter  + coding  region
       ==================================================
5851   TTTGGGCAAA AATTCCCGCA TTTTTTGTAG ATCAAACCGT AATGGGACAG
       AAACCCGTTT TTAAGGGCGT AAAAAACATC TAGTTTGGCA TTACCCTGTC

       ctl-1 promoter  + coding  region
       ==================================================
                                                     Exon 2
                                            ===================
5901   TCTGGCACCA CGTGACTATA TATTTTTAGC GGTCAACGAC ACAAAACCCG
       AGACCGTGGT GCACTGATAT ATAAAAATCG CCAGTTGCTG TGTTTTGGGC
```

Fig. 17 continued

```
          ctl-1 promoter  + coding  region
          ===============================================
                    Exon 2
          =============================================
5951      GACCAATGGC TGAGGATCAG CTGAAAGCTT ATAGAGATAG AAATCAGGTG
          CTGGTTACCG ACTCCTAGTC GACTTTCGAA TATCTCTATC TTTAGTCCAC

          ctl-1 promoter  + coding  region
          ===============================================
6001      AGAAAAATCA ATTTCAGCGA TTTTCTTCGC AATTTATATA AAAACTGATT
          TCTTTTTAGT TAAAGTCGCT AAAAGAAGCG TTAAATATAT TTTTGACTAA

          ctl-1 promoter  + coding  region
          =======================================
                                  o-GQ4
                        =========================
                             Exon 3
                ==================================
                                              SacI
                                             ~~~~~~
6051      TTTCCAGGAA CCCCACCTGC TCACCACATC CAATCGGAGC TCAGAAAAA
          AAAGGTCCTT GGGGTGGACG AGTGGTGTAG GTTAGCCTCG AGTCTTTTT
```

Fig. 18

Sod-3

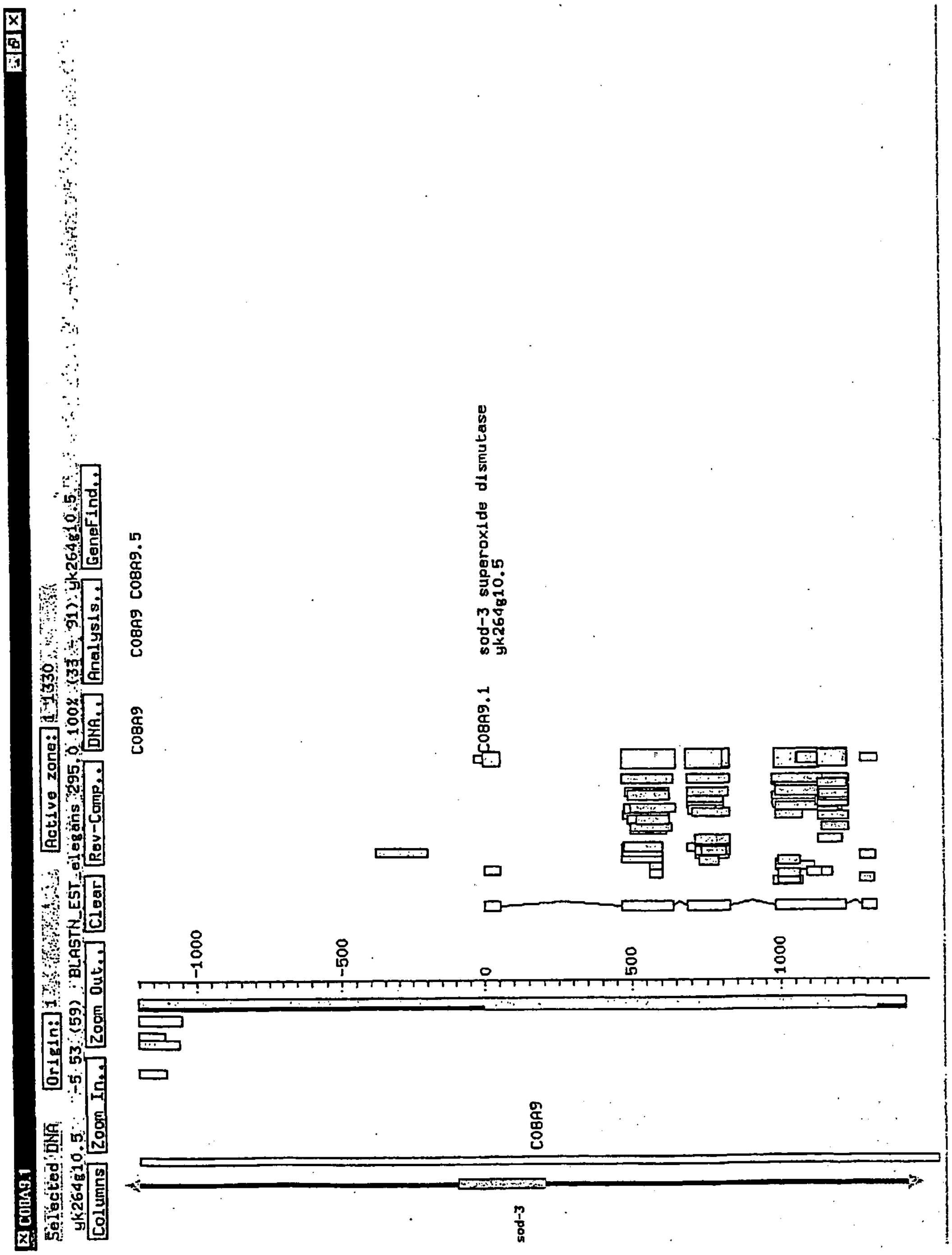

C0BA9.1
Selected DNA
Origin:
Active zone:
yk264g10.5
BLASTN_EST_elegans
yk264g10.5
Columns
Zoom In..
Zoom Out..
Clear
Rev-Comp..
DNA..
Analysis..
GeneFind..
C0BA9
C0BA9 C0BA9.5
C0BA9.1
sod-3 superoxide dismutase
yk264g10.5
-1000
-500
0
500
1000
C0BA9
sod-3

Figure 19

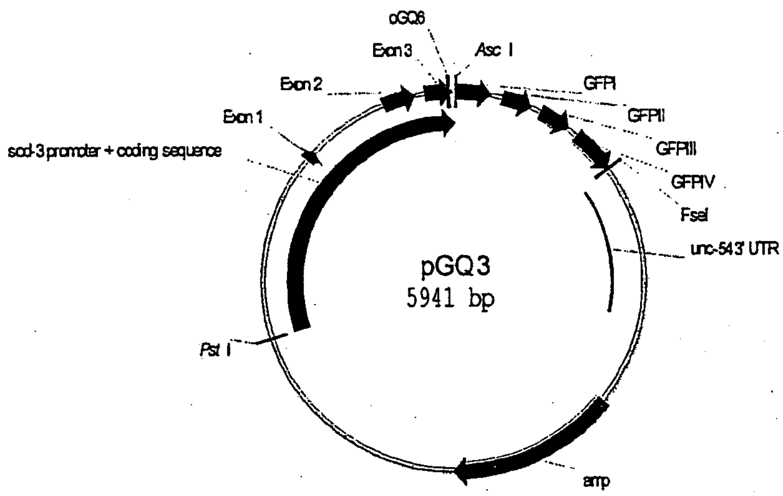

oGQ6
Exon 3
Asc I
Exon 2
GFPI
GFPII
Exon 1
GFPIII
sod-3 promoter + coding sequence
GFPIV
FseI
unc-54 3' UTR
pGQ3
5941 bp
Pst I
amp

Fig. 20

I. Predicted DNA sequence

```
    oGQ6                                              GFPI
    ==================================================
    AscI
    ~~~~~~
  1 CGCGCCATGA GTAAAGGAGA AGAACTTTTC ACTGGAGTTG TCCCAATTCT
    GCGCGGTACT CATTTCCTCT TCTTGAAAAG TGACCTCAAC AGGGTTAAGA

                                              GFPI
    ==================================================
 51 TGTTGAATTA GATGGTGATG TTAATGGGCA CAAATTTTCT GTCAGTGGAG
    ACAACTTAAT CTACCACTAC AATTACCCGT GTTTAAAAGA CAGTCACCTC

    GFPI
    ==================================================
101 AGGGTGAAGG TGATGCAACA TACGGAAAAC TTACCCTTAA ATTTATTTGC
    TCCCACTTCC ACTACGTTGT ATGCCTTTTG AATGGGAATT TAAATAAACG

    GFPI
    ===========================
151 ACTACTGGAA AACTACCTGT TCCATGGGTA AGTTTAAACA TATATATACT
    TGATGACCTT TTGATGGACA AGGTACCCAT TCAAATTTGT ATATATATGA

                                                 GFPII
                                ==================
201 AACTAACCCT GATTATTTAA ATTTTCAGCC AACACTTGTC ACTACTTTCT
    TTGATTGGGA CTAATAAATT TAAAAGTCGG TTGTGAACAG TGATGAAAGA

                                                 GFPII
    ==================================================
251 GTTATGGTGT TCAATGCTTC TCGAGATACC CAGATCATAT GAAACGGCAT
    CAATACCACA AGTTACGAAG AGCTCTATGG GTCTAGTATA CTTTGCCGTA

       GFPII
    ==================================================
301 GACTTTTTCA AGAGTGCCAT GCCCGAAGGT TATGTACAGG AAAGAACTAT
    CTGAAAAAGT TCTCACGGTA CGGGCTTCCA ATACATGTCC TTTCTTGATA

    GFPII
    ================================
351 ATTTTTCAAA GATGACGGGA ACTACAAGAC ACGTAAGTTT AAACAGTTCG
    TAAAAAGTTT CTACTGCCCT TGATGTTCTG TGCATTCAAA TTTGTCAAGC

                                                GFPIII
                                          ============
401 GTACTAACTA ACCATACATA TTTAAATTTT CAGGTGCTGA AGTCAAGTTT
    CATGATTGAT TGGTATGTAT AAATTTAAAA GTCCACGACT TCAGTTCAAA

                                                GFPIII
    ==================================================
```

Fig. 20 continued

```
451  GAAGGTGATA CCCTTGTTAA TAGAATCGAG TTAAAAGGTA TTGATTTTAA
     CTTCCACTAT GGGAACAATT ATCTTAGCTC AATTTTCCAT AACTAAAATT

                    GFPIII
     ==================================================
501  AGAAGATGGA AACATTCTTG GACACAAATT GGAATACAAC TATAACTCAC
     TCTTCTACCT TTGTAAGAAC CTGTGTTTAA CCTTATGTTG ATATTGAGTG

     GFPIII
     ===============================================
551  ACAATGTATA CATCATGGCA GACAAACAAA AGAATGGAAT CAAAGTTGTA
     TGTTACATAT GTAGTACCGT CTGTTTGTTT TCTTACCTTA GTTTCAACAT

                                                    GFPIV
                                                       ==
601  AGTTTAAACT TGGACTTACT AACTAACGGA TTATATTTAA ATTTTCAGAA
     TCAAATTTGA ACCTGAATGA TTGATTGCCT AATATAAATT TAAAAGTCTT

                                                    GFPIV
     ==================================================
651  CTTCAAAATT AGACACAACA TTGAAGATGG AAGCGTTCAA CTAGCAGACC
     GAAGTTTTAA TCTGTGTTGT AACTTCTACC TTCGCAAGTT GATCGTCTGG

                                                    GFPIV
     ==================================================
701  ATTATCAACA AAATACTCCA ATTGGCGATG GCCCTGTCCT TTTACCAGAC
     TAATAGTTGT TTTATGAGGT TAACCGCTAC CGGGACAGGA AAATGGTCTG

                 GFPIV
     ==================================================
751  AACCATTACC TGTCCACACA ATCTGCCCTT TCGAAAGATC CCAACGAAAA
     TTGGTAATGG ACAGGTGTGT TAGACGGGAA AGCTTTCTAG GGTTGCTTTT

     GFPIV
     ==================================================
801  GAGAGACCAC ATGGTCCTTC TTGAGTTTGT AACAGCTGCT GGGATTACAC
     CTCTCTGGTG TACCAGGAAG AACTCAAACA TTGTCGACGA CCCTAATGTG

     GFPIV                           FseI
     ==================================
851  ATGGCATGGA TGAACTATAC AAATAGGGCC GGCCGAGCTC CGCATCGGCC
     TACCGTACCT ACTTGATATG TTTATCCCGG CCGGCTCGAG GCGTAGCCGG

                                           unc-54  3' UTR
                           ==================================
901  GCTGTCATCA GATCGCCATC TCGCGCCCGT GCCTCTGACT TCTAAGTCCA
     CGACAGTAGT CTAGCGGTAG AGCGCGGGCA CGGAGACTGA AGATTCAGGT

                                           unc-54  3' UTR
     ==================================================
951  ATTACTCTTC AACATCCCTA CATGCTCTTT CTCCCTGTGC TCCCACCCCC
     TAATGAGAAG TTGTAGGGAT GTACGAGAAA GAGGGACACG AGGGTGGGGG

                                           unc-54  3' UTR
```

fig. 20 continued

```
     ==================================================
1001 TATTTTTGTT ATTATCAAAA AAACTTCTTC TTAATTTCTT TGTTTTTTAG
     ATAAAAACAA TAATAGTTTT TTTGAAGAAG AATTAAAGAA ACAAAAAATC

                                            unc-54  3' UTR
     ==================================================
1051 CTTCTTTTAA GTCACCTCTA ACAATGAAAT TGTGTAGATT CAAAAATAGA
     GAAGAAAATT CAGTGGAGAT TGTTACTTTA ACACATCTAA GTTTTTATCT

                                            unc-54  3' UTR
     ==================================================
1101 ATTAATTCGT AATAAAAAGT CGAAAAAAAT TGTGCTCCCT CCCCCCATTA
     TAATTAAGCA TTATTTTTCA GCTTTTTTTA ACACGAGGGA GGGGGGTAAT

                                            unc-54  3' UTR
     ==================================================
1151 ATAATAATTC TATCCCAAAA TCTACACAAT GTTCTGTGTA CACTTCTTAT
     TATTATTAAG ATAGGGTTTT AGATGTGTTA CAAGACACAT GTGAAGAATA

                                            unc-54  3' UTR
     ==================================================
1201 GTTTTTTTTA CTTCTGATAA ATTTTTTTTG AAACATCATA GAAAAAAACCG
     CAAAAAAAAT GAAGACTATT TAAAAAAAAC TTTGTAGTAT CTTTTTTGGC

                                 unc-54  3' UTR
     ==================================================
1251 CACACAAAAT ACCTTATCAT ATGTTACGTT TCAGTTTATG ACCGCAATTT
     GTGTGTTTTA TGGAATAGTA TACAATGCAA AGTCAAATAC TGGCGTTAAA

     unc-54 3'  UTR
     ==================================================
1301 TTATTTCTTC GCACGTCTGG GCCTCTCATG ACGTCAAATC ATGCTCATCG
     AATAAAGAAG CGTGCAGACC CGGAGAGTAC TGCAGTTTAG TACGAGTAGC

     unc-54 3'  UTR
     ==================================================
1351 TGAAAAAGTT TTGGAGTATT TTTGGAATTT TTCAATCAAG TGAAAGTTTA
     ACTTTTTCAA AACCTCATAA AAACCTTAAA AAGTTAGTTC ACTTTCAAAT

     unc-54 3'  UTR
     ==================================================
1401 TGAAATTAAT TTTCCTGCTT TTGCTTTTTG GGGGTTTCCC CTATTGTTTG
     ACTTTAATTA AAAGGACGAA AACGAAAAAC CCCCAAAGGG GATAACAAAC

     unc-54 3'  UTR
     ==================================================
1451 TCAAGAGTTT CGAGGACGGC GTTTTTCTTG CTAAAATCAC AAGTATTGAT
     AGTTCTCAAA GCTCCTGCCG CAAAAAGAAC GATTTTAGTG TTCATAACTA

     unc-54 3'  UTR
     ==================================================
1501 GAGCACGATG CAAGAAAGAT CGGAAGAAGG TTTGGGTTTG AGGCTCAGTG
     CTCGTGCTAC GTTCTTTCTA GCCTTCTTCC AAACCCAAAC TCCGAGTCAC
```

Fig. 20 continued

```
     unc-54 3'  UTR
     ==================================================
1551 GAAGGTGAGT AGAAGTTGAT AATTTGAAAG TGGAGTAGTG TCTATGGGGT
     CTTCCACTCA TCTTCAACTA TTAAACTTTC ACCTCATCAC AGATACCCCA

     unc-54 3'  UTR
     ==================================================
1601 TTTTGCCTTA AATGACAGAA TACATTCCCA ATATACCAAA CATAACTGTT
     AAAACGGAAT TTACTGTCTT ATGTAAGGGT TATATGGTTT GTATTGACAA

     unc-54 3'  UTR
     =
1651 TCCTACTAGT CGGCCGTACG GGCCCTTTCG TCTCGCGCGT TTCGGTGATG
     AGGATGATCA GCCGGCATGC CCGGGAAAGC AGAGCGCGCA AAGCCACTAC

1701 ACGGTGAAAA CCTCTGACAC ATGCAGCTCC CGGAGACGGT CACAGCTTGT
     TGCCACTTTT GGAGACTGTG TACGTCGAGG GCCTCTGCCA GTGTCGAACA

1751 CTGTAAGCGG ATGCCGGGAG CAGACAAGCC CGTCAGGGCG CGTCAGCGGG
     GACATTCGCC TACGGCCCTC GTCTGTTCGG GCAGTCCCGC GCAGTCGCCC

1801 TGTTGGCGGG TGTCGGGGCT GGCTTAACTA TGCGGCATCA GAGCAGATTG
     ACAACCGCCC ACAGCCCCGA CCGAATTGAT ACGCCGTAGT CTCGTCTAAC

1851 TACTGAGAGT GCACCATATG CGGTGTGAAA TACCGCACAG ATGCGTAAGG
     ATGACTCTCA CGTGGTATAC GCCACACTTT ATGGCGTGTC TACGCATTCC

1901 AGAAAATACC GCATCAGGCG GCCTTAAGGG CCTCGTGATA CGCCTATTTT
     TCTTTTATGG CGTAGTCCGC CGGAATTCCC GGAGCACTAT GCGGATAAAA

1951 TATAGGTTAA TGTCATGATA ATAATGGTTT CTTAGACGTC AGGTGGCACT
     ATATCCAATT ACAGTACTAT TATTACCAAA GAATCTGCAG TCCACCGTGA

2001 TTTCGGGGAA ATGTGCGCGG AACCCCTATT TGTTTATTTT TCTAAATACA
     AAAGCCCCTT TACACGCGCC TTGGGGATAA ACAAATAAAA AGATTTATGT

2051 TTCAAATATG TATCCGCTCA TGAGACAATA ACCCTGATAA ATGCTTCAAT
     AAGTTTATAC ATAGGCGAGT ACTCTGTTAT TGGGACTATT TACGAAGTTA

                                                       amp
                           ==============================
2101 AATATTGAAA AAGGAAGAGT ATGAGTATTC AACATTTCCG TGTCGCCCTT
     TTATAACTTT TTCCTTCTCA TACTCATAAG TTGTAAAGGC ACAGCGGGAA

                                                       amp
     ==================================================
2151 ATTCCCTTTT TTGCGGCATT TTGCCTTCCT GTTTTTGCTC ACCCAGAAAC
     TAAGGGAAAA AACGCCGTAA AACGGAAGGA CAAAAACGAG TGGGTCTTTG

                                                       amp
     ==================================================
2201 GCTGGTGAAA GTAAAAGATG CTGAAGATCA GTTGGGTGCA CGAGTGGGTT
     CGACCACTTT CATTTTCTAC GACTTCTAGT CAACCCACGT GCTCACCCAA
```

Fig. 20 continued

```
                                                          amp
     ======================================================
2251 ACATCGAACT GGATCTCAAC AGCGGTAAGA TCCTTGAGAG TTTTCGCCCC
     TGTAGCTTGA CCTAGAGTTG TCGCCATTCT AGGAACTCTC AAAAGCGGGG

                                                          amp
     ======================================================
2301 GAAGAACGTT TTCCAATGAT GAGCACTTTT AAAGTTCTGC TATGTGGCGC
     CTTCTTGCAA AAGGTTACTA CTCGTGAAAA TTTCAAGACG ATACACCGCG

                                                          amp
     ======================================================
2351 GGTATTATCC CGTATTGACG CCGGGCAAGA GCAACTCGGT CGCCGCATAC
     CCATAATAGG GCATAACTGC GGCCCGTTCT CGTTGAGCCA GCGGCGTATG

                                                          amp
     ======================================================
2401 ACTATTCTCA GAATGACTTG GTTGAGTACT CACCAGTCAC AGAAAAGCAT
     TGATAAGAGT CTTACTGAAC CAACTCATGA GTGGTCAGTG TCTTTTCGTA

                                                          amp
     ======================================================
2451 CTTACGGATG GCATGACAGT AAGAGAATTA TGCAGTGCTG CCATAACCAT
     GAATGCCTAC CGTACTGTCA TTCTCTTAAT ACGTCACGAC GGTATTGGTA

                                                          amp
     ======================================================
2501 GAGTGATAAC ACTGCGGCCA ACTTACTTCT GACAACGATC GGAGGACCGA
     CTCACTATTG TGACGCCGGT TGAATGAAGA CTGTTGCTAG CCTCCTGGCT

     amp
     ======================================================
2551 AGGAGCTAAC CGCTTTTTTG CACAACATGG GGGATCATGT AACTCGCCTT
     TCCTCGATTG GCGAAAAAAC GTGTTGTACC CCCTAGTACA TTGAGCGGAA

     amp
     ======================================================
2601 GATCGTTGGG AACCGGAGCT GAATGAAGCC ATACCAAACG ACGAGCGTGA
     CTAGCAACCC TTGGCCTCGA CTTACTTCGG TATGGTTTGC TGCTCGCACT

     amp
     ======================================================
2651 CACCACGATG CCTGTAGCAA TGGCAACAAC GTTGCGCAAA CTATTAACTG
     GTGGTGCTAC GGACATCGTT ACCGTTGTTG CAACGCGTTT GATAATTGAC

     amp
     ======================================================
2701 GCGAACTACT TACTCTAGCT TCCCGGCAAC AATTAATAGA CTGGATGGAG
     CGCTTGATGA ATGAGATCGA AGGGCCGTTG TTAATTATCT GACCTACCTC

     amp
     ======================================================
2751 GCGGATAAAG TTGCAGGACC ACTTCTGCGC TCGGCCCTTC CGGCTGGCTG
     CGCCTATTTC AACGTCCTGG TGAAGACGCG AGCCGGGAAG GCCGACCGAC
```

Fig. 20 continued

```
     amp
     ==========================================================
2801 GTTTATTGCT GATAAATCTG GAGCCGGTGA GCGTGGGTCT CGCGGTATCA
     CAAATAACGA CTATTTAGAC CTCGGCCACT CGCACCCAGA GCGCCATAGT

     amp
     ==========================================================
2851 TTGCAGCACT GGGGCCAGAT GGTAAGCCCT CCCGTATCGT AGTTATCTAC
     AACGTCGTGA CCCCGGTCTA CCATTCGGGA GGGCATAGCA TCAATAGATG

     amp
     ==========================================================
2901 ACGACGGGGA GTCAGGCAAC TATGGATGAA CGAAATAGAC AGATCGCTGA
     TGCTGCCCCT CAGTCCGTTG ATACCTACTT GCTTTATCTG TCTAGCGACT

     amp
     ================================
2951 GATAGGTGCC TCACTGATTA AGCATTGGTA ACTGTCAGAC CAAGTTTACT
     CTATCCACGG AGTGACTAAT TCGTAACCAT TGACAGTCTG GTTCAAATGA

3001 CATATATACT TTAGATTGAT TTAAAACTTC ATTTTTAATT TAAAAGGATC
     GTATATATGA AATCTAACTA AATTTTGAAG TAAAAATTAA ATTTTCCTAG

3051 TAGGTGAAGA TCCTTTTTGA TAATCTCATG ACCAAAATCC CTTAACGTGA
     ATCCACTTCT AGGAAAAACT ATTAGAGTAC TGGTTTTAGG GAATTGCACT

3101 GTTTTCGTTC CACTGAGCGT CAGACCCCGT AGAAAAGATC AAAGGATCTT
     CAAAAGCAAG GTGACTCGCA GTCTGGGGCA TCTTTTCTAG TTTCCTAGAA

3151 CTTGAGATCC TTTTTTTCTG CGCGTAATCT GCTGCTTGCA AACAAAAAAA
     GAACTCTAGG AAAAAAAGAC GCGCATTAGA CGACGAACGT TTGTTTTTTT

3201 CCACCGCTAC CAGCGGTGGT TTGTTTGCCG GATCAAGAGC TACCAACTCT
     GGTGGCGATG GTCGCCACCA AACAAACGGC CTAGTTCTCG ATGGTTGAGA

3251 TTTTCCGAAG GTAACTGGCT TCAGCAGAGC GCAGATACCA AATACTGTCC
     AAAAGGCTTC CATTGACCGA AGTCGTCTCG CGTCTATGGT TTATGACAGG

3301 TTCTAGTGTA GCCGTAGTTA GGCCACCACT TCAAGAACTC TGTAGCACCG
     AAGATCACAT CGGCATCAAT CCGGTGGTGA AGTTCTTGAG ACATCGTGGC

3351 CCTACATACC TCGCTCTGCT AATCCTGTTA CCAGTGGCTG CTGCCAGTGG
     GGATGTATGG AGCGAGACGA TTAGGACAAT GGTCACCGAC GACGGTCACC

3401 CGATAAGTCG TGTCTTACCG GGTTGGACTC AAGACGATAG TTACCGGATA
     GCTATTCAGC ACAGAATGGC CCAACCTGAG TTCTGCTATC AATGGCCTAT

3451 AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT CGTGCACACA GCCCAGCTTG
     TCCGCGTCGC CAGCCCGACT TGCCCCCCAA GCACGTGTGT CGGGTCGAAC

3501 GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG AGCATTGAGA
     CTCGCTTGCT GGATGTGGCT TGACTCTATG GATGTCGCAC TCGTAACTCT
```

Fig. 70 continued

```
3551  AAGCGCCACG CTTCCCGAAG GGAGAAAGGC GGACAGGTAT CCGGTAAGCG
      TTCGCGGTGC GAAGGGCTTC CCTCTTTCCG CCTGTCCATA GGCCATTCGC

3601  GCAGGGTCGG AACAGGAGAG CGCACGAGGG AGCTTCCAGG GGGAAACGCC
      CGTCCCAGCC TTGTCCTCTC GCGTGCTCCC TCGAAGGTCC CCCTTTGCGG

3651  TGGTATCTTT ATAGTCCTGT CGGGTTTCGC CACCTCTGAC TTGAGCGTCG
      ACCATAGAAA TATCAGGACA GCCCAAAGCG GTGGAGACTG AACTCGCAGC

3701  ATTTTTGTGA TGCTCGTCAG GGGGGCGGAG CCTATGGAAA AACGCCAGCA
      TAAAAACACT ACGAGCAGTC CCCCCGCCTC GGATACCTTT TTGCGGTCGT

3751  ACGCGGCCTT TTTACGGTTC CTGGCCTTTT GCTGGCCTTT TGCTCACATG
      TGCGCCGGAA AAATGCCAAG GACCGGAAAA CGACCGGAAA ACGAGTGTAC

3801  TTCTTTCCTG CGTTATCCCC TGATTCTGTG GATAACCGTA TTACCGCCTT
      AAGAAAGGAC GCAATAGGGG ACTAAGACAC CTATTGGCAT AATGGCGGAA

3851  TGAGTGAGCT GATACCGCTC GCCGCAGCCG AACGACCGAG CGCAGCGAGT
      ACTCACTCGA CTATGGCGAG CGGCGTCGGC TTGCTGGCTC GCGTCGCTCA

3901  CAGTGAGCGA GGAAGCGGAA GAGCGCCCAA TACGCAAACC GCCTCTCCCC
      GTCACTCGCT CCTTCGCCTT CTCGCGGGTT ATGCGTTTGG CGGAGAGGGG

3951  GCGCGTTGGC CGATTCATTA ATGCAGCTGG CACGACAGGT TTCCCGACTG
      CGCGCAACCG GCTAAGTAAT TACGTCGACC GTGCTGTCCA AAGGGCTGAC

4001  GAAAGCGGGC AGTGAGCGCA ACGCAATTAA TGTGAGTTAG CTCACTCATT
      CTTTCGCCCG TCACTCGCGT TGCGTTAATT ACACTCAATC GAGTGAGTAA

4051  AGGCACCCCA GGCTTTACAC TTTATGCTTC CGGCTCGTAT GTTGTGTGGA
      TCCGTGGGGT CCGAAATGTG AAATACGAAG GCCGAGCATA CAACACACCT

4101  ATTGTGAGCG GATAACAATT TCACACAGGA AACAGCTATG ACCATGATTA
      TAACACTCGC CTATTGTTAA AGTGTGTCCT TTGTCGATAC TGGTACTAAT

                              sod-3 promoter + coding sequence
                                   ==========================
                           PstI
                          ~~~~~~~
4151  CGCCAAGCTT GCATGCCTGC AGTGATTCAG AGAGGTTGAG AATTATTTTC
      GCGGTTCGAA CGTACGGACG TCACTAAGTC TCTCCAACTC TTAATAAAAG

                              sod-3 promoter + coding sequence
      =======================================================
4201  AAAAACATTC AATGTTTTCC CTTGGAGTGA CTATGCAAAT ATGAAAATGT
      TTTTTGTAAG TTACAAAAGG GAACCTCACT GATACGTTTA TACTTTTACA

                              sod-3 promoter + coding sequence
      =======================================================
4251  TTTCCAAAAA TATTTGGATG CCCTGATAAA AAGTAGGTGA AATTTCGCAG
      AAAGGTTTTT ATAAACCTAC GGGACTATTT TTCATCCACT TTAAAGCGTC

                              sod-3 promoter + coding sequence
```

Fig. 20 continued

```
     ==================================================
4301 GGGAACATCA TATTAAAATG TTGAATTTTT AGAAGAAATG GAAATGTTTG
     CCCTTGTAGT ATAATTTTAC AACTTAAAAA TCTTCTTTAC CTTTACAAAC

                          sod-3 promoter + coding sequence
     ==================================================
4351 TCGGTGGTAT GCTCGAATAT TTGAGATATT ATATATTTAC TGTTAAATCC
     AGCCACCATA CGAGCTTATA AACTCTATAA TATATAAATG ACAATTTAGG

                          sod-3 promoter + coding sequence
     ==================================================
4401 GAAATTTTTG ACAAACGGAA AAAATTTGTG TCGAAATACT ACATTTTCGA
     CTTTAAAAAC TGTTTGCCTT TTTTAAACAC AGCTTTATGA TGTAAAAGCT

                          sod-3 promoter + coding sequence
     ==================================================
4451 TAACACAAAG GTACTTCCAT AACACTTATA AAAACTGTTT GACTATCTTA
     ATTGTGTTTC CATGAAGGTA TTGTGAATAT TTTTGACAAA CTGATAGAAT

                          sod-3 promoter + coding sequence
     ==================================================
4501 TTTCAGGAAA AAAAAATCCA AGAATAAACA TTTTTCAGAA TTTGAACTTT
     AAAGTCCTTT TTTTTTAGGT TCTTATTTGT AAAAAGTCTT AAACTTGAAA

                          sod-3 promoter + coding sequence
     ==================================================
4551 CTAATGGCTG ATTAATAAAA CAAAGTTATA CAACTATTCA AAGCAGTTGC
     GATTACCGAC TAATTATTTT GTTTCAATAT GTTGATAAGT TTCGTCAACG

                          sod-3 promoter + coding sequence
     ==================================================
4601 TCAATCTGGC ATTTTCTTGT GTTTTTTTTT GAATATTTCA TCAGCAAGAT
     AGTTAGACCG TAAAAGAACA CAAAAAAAAA CTTATAAAGT AGTCGTTCTA

                          sod-3 promoter + coding sequence
     ==================================================
4651 GTTGATAATT TTGTGTTAAT TCTAATTGTT TTCTACAATT TTTCAAACCG
     CAACTATTAA AACACAATTA AGATTAACAA AAGATGTTAA AAAGTTTGGC

                          sod-3 promoter + coding sequence
     ==================================================
4701 AAAATTGACC TTTGACTTTG TTTACTTTGT TCTCGTGGGT TAACTGTTCA
     TTTTAACTGG AAACTGAAAC AAATGAAACA AGAGCACCCA ATTGACAAGT

                          sod-3 promoter + coding sequence
     ==================================================
4751 CTGATTTCTA TTGCTGTTGA TGAGGTCTTT GATCAAATTT GTATTGTTTT
     GACTAAAGAT AACGACAACT ACTCCAGAAA CTAGTTTAAA CATAACAAAA

                          sod-3 promoter + coding sequence
     ==================================================
4801 TATACTGCAT ATTGCTTCAA TTCTAAATCA TCTAATATAT TGTCAAACAA
     ATATGACGTA TAACGAAGTT AAGATTTAGT AGATTATATA ACAGTTTGTT
```

Fig. 20 continued

```
                         sod-3  promoter  + coding  sequence
     ==========================================================
4851 CTTCTTGTTT TTTTTTTCAT TCAAAACTTC TGCAAAAACG TTCTCTTAAC
     GAAGAACAAA AAAAAAAGTA AGTTTTGAAG ACGTTTTTGC AAGAGAATTG

                         sod-3  promoter  + coding  sequence
     ==========================================================
4901 AAAGGTTCAC ACAACAACTC TCCTCTCCAT CTCTTTCTCT CAACAACAAT
     TTTCCAAGTG TGTTGTTGAG AGGAGAGGTA GAGAAAGAGA GTTGTTGTTA

                         sod-3  promoter  + coding  sequence
     ==========================================================
4951 GTGCTGGCCT TGCATGTTTG CCAGTGCGGG TTGTTTACGC GTTTTCAAGA
     CACGACCGGA ACGTACAAAC GGTCACGCCC AACAAATGCG CAAAAGTTCT

                         sod-3  promoter  + coding  sequence
     ==========================================================
5001 TTTTTGGTCT CCTATCTAAC GTCCCGAAAT GCATTTTTTC CTTTCATTTG
     AAAAACCAGA GGATAGATTG CAGGGCTTTA CGTAAAAAAG GAAAGTAAAC

     sod-3 promoter  + coding  sequence
     ==========================================================
5051 GTTTTTTTCT GTTCGAGAAA AGTGACCGTT TGTCAAATCT TCTAATTTTC
     CAAAAAAAGA CAAGCTCTTT TCACTGGCAA ACAGTTTAGA AGATTAAAAG

     sod-3 promoter  + coding  sequence
     ==========================================================
                                             Exon 1
                ===========================================
5101 AGTGAATAAA ATGCTGCAAT CTACTGCTCG CACTGCTTCA AAGCTTGTTC
     TCACTTATTT TACGACGTTA GATGACGAGC GTGACGAAGT TTCGAACAAG

     sod-3 promoter  + coding  sequence
     ==========================================================
     Exon 1
     ==============
5151 AACCGGTTGC GGGGTAAGTC AAAATGAAAT TTTCGTTTAA AAATTGGTTT
     TTGGCCAACG CCCCATTCAG TTTTACTTTA AAAGCAAATT TTTAACCAAA

     sod-3 promoter  + coding  sequence
     ==========================================================
5201 TTTTTGGTAT TATAGATAAA ACTTATACCA AAACAAAACA TATTTAGAAA
     AAAAACCATA ATATCTATTT TGAATATGGT TTTGTTTTGT ATAAATCTTT

     sod-3 promoter  + coding  sequence
     ==========================================================
5251 AACTTTAATA GAGAATAATT GTTTAATAAT TAATTTTTGC AAGCTCCTTT
     TTGAAATTAT CTCTTATTAA CAAATTATTA ATTAAAAACG TTCGAGGAAA

     sod-3 promoter  + coding  sequence
     ==========================================================
5301 TAAATTAAGA CATCTAAAAC AGTTTTCAGC TTGATTGTTT TAATGGTTTA
     ATTTAATTCT GTAGATTTTG TCAAAAGTCG AACTAACAAA ATTACCAAAT
```

Fig. 20 continued

```
      sod-3 promoter  + coding  sequence
      ==================================================
5351  GAAAGCAATA TTTGTATTTT GTGTTAAACT GAAAATATCT AGGAAATACT
      CTTTCGTTAT AAACATAAAA CACAATTTGA CTTTTATAGA TCCTTTATGA

      sod-3 promoter  + coding  sequence
      ==================================================
5401  ACTTTTAAAA TATTTGAAAC TTGAAATTTT AAAATTCCAA ATAATTTTAC
      TGAAAATTTT ATAAACTTTG AACTTTAAAA TTTTAAGGTT TATTAAAATG

      sod-3 promoter  + coding  sequence
      ==================================================
5451  TCATTTCCTA AAGTGTTTGA GTATTTGTAT CCTGTGCTGA CACCGAAATG
      AGTAAAGGAT TTCACAAACT CATAAACATA GGACACGACT GTGGCTTTAC

      sod-3 promoter  + coding  sequence
      ==================================================
5501  TTCTCAATTT TGGAAAAAAA AGATTTTTAT CCGTATCTTC AGTCTTACAA
      AAGAGTTAAA ACCTTTTTTT TCTAAAAATA GGCATAGAAG TCAGAATGTT

      sod-3 promoter  + coding  sequence
      ==================================================
                                                  Exon 2
                                        ==================
5551  TTTTTTTCAC CTTTTTTTTC ATTTCAGAGT TCTCGCCGTC CGCTCCAAGC
      AAAAAAAGTG GAAAAAAAAG TAAAGTCTCA AGAGCGGCAG GCGAGGTTCG

      sod-3 promoter  + coding  sequence
      ==================================================
                                                  Exon 2
      ==================================================
5601  ACACTCTCCC AGATCTCCCA TTCGACTATG CAGATTTGGA ACCTGTAATC
      TGTGAGAGGG TCTAGAGGGT AAGCTGATAC GTCTAAACCT TGGACATTAG

      sod-3 promoter  + coding  sequence
      ==================================================
                 Exon 2
      ==================================================
5651  AGCCATGAAA TCATGCAGCT TCATCATCAA AAGCATCATG CCACCTACGT
      TCGGTACTTT AGTACGTCGA AGTAGTAGTT TTCGTAGTAC GGTGGATGCA

      sod-3 promoter  + coding  sequence
      ==================================================
      Exon 2
      ==================================================
5701  GAACAATCTC AATCAGATCG AGGAGAAACT TCACGAGGCT GTTTCGAAAG
      CTTGTTAGAG TTAGTCTAGC TCCTCTTTGA AGTGCTCCGA CAAAGCTTTC

      sod-3 promoter  + coding  sequence
      ==================================================
                                                  Exon 3
                                                      ==
5751  GTTTTTTAAT CAGAAGATTT TGAAATGAAT TTTTTTTTTG GTATATAGGG
      CAAAAAATTA GTCTTCTAAA ACTTTACTTA AAAAAAAAAC CATATATCCC
```

Fig. 20 continued

```
     sod-3 promoter  + coding  sequence
     =====================================================
                                                   Exon 3
     =====================================================
5801 AATCTAAAAG AAGCAATTGC TCTCCAACCA GCGCTGAAAT TCAATGGTGG
     TTAGATTTTC TTCGTTAACG AGAGGTTGGT CGCGACTTTA AGTTACCACC

     sod-3 promoter  + coding  sequence
     =====================================================
                   Exon 3
     =====================================================
5851 TGGACACATC AATCATTCTA TCTTCTGGAC CAACTTGGCT AAGGATGGTG
     ACCTGTGTAG TTAGTAAGAT AGAAGACCTG GTTGAACCGA TTCCTACCAC

                                   oGQ6
                     ==========================
     sod-3 promoter  + coding  sequence
     ==========================================
     Exon 3
     ====================================
                                            AscI
                                              ~~
5901 GAGAACCTTC AAAGGAGCTG ATGGACACTA TTAAGGCTTG G
     CTCTTGGAAG TTTCCTCGAC TACCTGTGAT AATTCCGAAC C
```

Figure 21

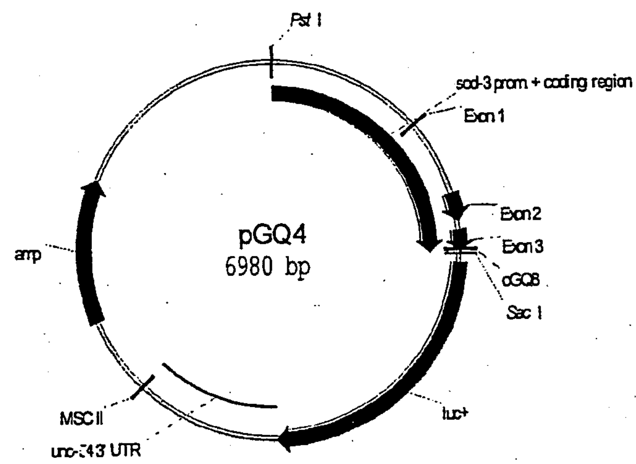

Pst I
sod-3 prom + coding region
Exon 1
Exon 2
Exon 3
oGQ8
Sac I
luc+
unc-54 3' UTR
MSC II
amp
pGQ4
6980 bp

Fig. 22

II. Predicted DNA sequence

```
                                  sod-3 prom.  + coding  region
    ============================================================
    PstI
    ~
  1 GTGATTCAGA GAGGTTGAGA ATTATTTTCA AAAACATTCA ATGTTTTCCC
    CACTAAGTCT CTCCAACTCT TAATAAAAGT TTTTGTAAGT TACAAAAGGG

                                  sod-3 prom.  + coding  region
    ============================================================
 51 TTGGAGTGAC TATGCAAATA TGAAAATGTT TTCCAAAAAT ATTTGGATGC
    AACCTCACTG ATACGTTTAT ACTTTTACAA AAGGTTTTTA TAAACCTACG

                                  sod-3 prom.  + coding  region
    ============================================================
101 CCTGATAAAA AGTAGGTGAA ATTTCGCAGG GGAACATCAT ATTAAAATGT
    GGACTATTTT TCATCCACTT TAAAGCGTCC CCTTGTAGTA TAATTTTACA

                                  sod-3 prom.  + coding  region
    ============================================================
151 TGAATTTTTA GAAGAAATGG AAATGTTTGT CGGTGGTATG CTCGAATATT
    ACTTAAAAAT CTTCTTTACC TTTACAAACA GCCACCATAC GAGCTTATAA

                                  sod-3 prom.  + coding  region
    ============================================================
201 TGAGATATTA TATATTTACT GTTAAATCCG AAATTTTTGA CAAACGGAAA
    ACTCTATAAT ATATAAATGA CAATTTAGGC TTTAAAAACT GTTTGCCTTT

                                  sod-3 prom.  + coding  region
    ============================================================
251 AAATTTGTGT CGAAATACTA CATTTTCGAT AACACAAAGG TACTTCCATA
    TTTAAACACA GCTTTATGAT GTAAAAGCTA TTGTGTTTCC ATGAAGGTAT

                                  sod-3 prom.  + coding  region
    ============================================================
301 ACACTTATAA AAACTGTTTG ACTATCTTAT TTCAGGAAAA AAAAATCCAA
    TGTGAATATT TTTGACAAAC TGATAGAATA AAGTCCTTTT TTTTTAGGTT

                                  sod-3 prom.  + coding  region
    ============================================================
351 GAATAAACAT TTTTCAGAAT TTGAACTTTC TAATGGCTGA TTAATAAAAC
    CTTATTTGTA AAAAGTCTTA AACTTGAAAG ATTACCGACT AATTATTTTG

                                  sod-3 prom.  + coding  region
    ============================================================
401 AAAGTTATAC AACTATTCAA AGCAGTTGCT CAATCTGGCA TTTTCTTGTG
    TTTCAATATG TTGATAAGTT TCGTCAACGA GTTAGACCGT AAAAGAACAC

                                  sod-3 prom.  + coding  region
    ============================================================
```

Fig. 22 continued

```
451  TTTTTTTTTG AATATTTCAT CAGCAAGATG TTGATAATTT TGTGTTAATT
     AAAAAAAAAC TTATAAAGTA GTCGTTCTAC AACTATTAAA ACACAATTAA

                               sod-3 prom.  + coding  region
     ==================================================
501  CTAATTGTTT TCTACAATTT TTCAAACCGA AAATTGACCT TTGACTTTGT
     GATTAACAAA AGATGTTAAA AAGTTTGGCT TTTAACTGGA AACTGAAACA

                               sod-3 prom.  + coding  region
     ==================================================
551  TTACTTTGTT CTCGTGGGTT AACTGTTCAC TGATTTCTAT TGCTGTTGAT
     AATGAAACAA GAGCACCCAA TTGACAAGTG ACTAAAGATA ACGACAACTA

                               sod-3 prom.  + coding  region
     ==================================================
601  GAGGTCTTTG ATCAAATTTG TATTGTTTTT ATACTGCATA TTGCTTCAAT
     CTCCAGAAAC TAGTTTAAAC ATAACAAAAA TATGACGTAT AACGAAGTTA

                               sod-3 prom.  + coding  region
     ==================================================
651  TCTAAATCAT CTAATATATT GTCAAACAAC TTCTTGTTTT TTTTTTCATT
     AGATTTAGTA GATTATATAA CAGTTTGTTG AAGAACAAAA AAAAAAGTAA

                               sod-3 prom.  + coding  region
     ==================================================
701  CAAAACTTCT GCAAAAACGT TCTCTTAACA AAGGTTCACA CAACAACTCT
     GTTTTGAAGA CGTTTTTGCA AGAGAATTGT TTCCAAGTGT GTTGTTGAGA

                               sod-3 prom.  + coding  region
     ==================================================
751  CCTCTCCATC TCTTTCTCTC AACAACAATG TGCTGGCCTT GCATGTTTGC
     GGAGAGGTAG AGAAAGAGAG TTGTTGTTAC ACGACCGGAA CGTACAAACG

                               sod-3 prom.  + coding  region
     ==================================================
801  CAGTGCGGGT TGTTTACGCG TTTTCAAGAT TTTTGGTCTC CTATCTAACG
     GTCACGCCCA ACAAATGCGC AAAAGTTCTA AAAACCAGAG GATAGATTGC

                               sod-3 prom.  + coding  region
     ==================================================
851  TCCCGAAATG CATTTTTTCC TTTCATTTGG TTTTTTTCTG TTCGAGAAAA
     AGGGCTTTAC GTAAAAAAGG AAAGTAAACC AAAAAAAGAC AAGCTCTTTT

     sod-3 prom.  + coding  region
     ==================================================
                                                  Exon 1
                                             ==========
901  GTGACCGTTT GTCAAATCTT CTAATTTTCA GTGAATAAAA TGCTGCAATC
     CACTGGCAAA CAGTTTAGAA GATTAAAAGT CACTTATTTT ACGACGTTAG

     sod-3 prom.  + coding  region
     ==================================================
            Exon 1
     ==========================================
```

Fig. 22 continued

```
951   TACTGCTCGC ACTGCTTCAA AGCTTGTTCA ACCGGTTGCG GGGTAAGTCA
      ATGACGAGCG TGACGAAGTT TCGAACAAGT TGGCCAACGC CCCATTCAGT

      sod-3 prom.  + coding  region
      ==================================================
1001  AAATGAAATT TTCGTTTAAA AATTGGTTTT TTTTGGTATT ATAGATAAAA
      TTTACTTTAA AAGCAAATTT TTAACCAAAA AAAACCATAA TATCTATTTT

      sod-3 prom.  + coding  region
      ==================================================
1051  CTTATACCAA AACAAAACAT ATTTAGAAAA ACTTTAATAG AGAATAATTG
      GAATATGGTT TTGTTTTGTA TAAATCTTTT TGAAATTATC TCTTATTAAC

      sod-3 prom.  + coding  region
      ==================================================
1101  TTTAATAATT AATTTTTGCA AGCTCCTTTT AAATTAAGAC ATCTAAAACA
      AAATTATTAA TTAAAAACGT TCGAGGAAAA TTTAATTCTG TAGATTTTGT

      sod-3 prom.  + coding  region
      ==================================================
1151  GTTTTCAGCT TGATTGTTTT AATGGTTTAG AAAGCAATAT TTGTATTTTG
      CAAAAGTCGA ACTAACAAAA TTACCAAATC TTTCGTTATA AACATAAAAC

      sod-3 prom.  + coding  region
      ==================================================
1201  TGTTAAACTG AAAATATCTA GGAAATACTA CTTTTAAAAT ATTTGAAACT
      ACAATTTGAC TTTTATAGAT CCTTTATGAT GAAAATTTTA TAAACTTTGA

      sod-3 prom.  + coding  region
      ==================================================
1251  TGAAATTTTA AAATTCCAAA TAATTTTACT CATTTCCTAA AGTGTTTGAG
      ACTTTAAAAT TTTAAGGTTT ATTAAAATGA GTAAAGGATT TCACAAACTC

      sod-3 prom.  + coding  region
      ==================================================
1301  TATTTGTATC CTGTGCTGAC ACCGAAATGT TCTCAATTTT GGAAAAAAAA
      ATAAACATAG GACACGACTG TGGCTTTACA AGAGTTAAAA CCTTTTTTTT

      sod-3 prom.  + coding  region
      ==================================================
1351  GATTTTTATC CGTATCTTCA GTCTTACAAT TTTTTTCACC TTTTTTTTCA
      CTAAAAATAG GCATAGAAGT CAGAATGTTA AAAAAAGTGG AAAAAAAAGT

      sod-3 prom.  + coding  region
      ==================================================
                                                    Exon 2
              ==========================================
1401  TTTCAGAGTT CTCGCCGTCC GCTCCAAGCA CACTCTCCCA GATCTCCCAT
      AAAGTCTCAA GAGCGGCAGG CGAGGTTCGT GTGAGAGGGT CTAGAGGGTA

      sod-3 prom.  + coding  region
      ==================================================
                                                Exon  2
      ==================================================
```

fig. 22 continued

```
1451  TCGACTATGC AGATTTGGAA CCTGTAATCA GCCATGAAAT CATGCAGCTT
      AGCTGATACG TCTAAACCTT GGACATTAGT CGGTACTTTA GTACGTCGAA

      sod-3 prom.  + coding  region
      =====================================================
      Exon 2
      =====================================================
1501  CATCATCAAA AGCATCATGC CACCTACGTG AACAATCTCA ATCAGATCGA
      GTAGTAGTTT TCGTAGTACG GTGGATGCAC TTGTTAGAGT TAGTCTAGCT

      sod-3 prom.  + coding  region
      =====================================================
      Exon 2
      ==============================
1551  GGAGAAACTT CACGAGGCTG TTTCGAAAGG TTTTTTAATC AGAAGATTTT
      CCTCTTTGAA GTGCTCCGAC AAAGCTTTCC AAAAAATTAG TCTTCTAAAA

      sod-3 prom.  + coding  region
      =====================================================
                                                     Exon 3
                                    ========================
1601  GAAATGAATT TTTTTTTTGG TATATAGGGA ATCTAAAAGA AGCAATTGCT
      CTTTACTTAA AAAAAAAACC ATATATCCCT TAGATTTTCT TCGTTAACGA

      sod-3 prom.  + coding  region
      =====================================================
                                                   Exon 3
      =====================================================
1651  CTCCAACCAG CGCTGAAATT CAATGGTGGT GGACACATCA ATCATTCTAT
      GAGGTTGGTC GCGACTTTAA GTTACCACCA CCTGTGTAGT TAGTAAGATA

                                                       oGQ8
                                                      =====
      sod-3 prom.  + coding  region
      =====================================================
      Exon 3
      =====================================================
1701  CTTCTGGACC AACTTGGCTA AGGATGGTGG AGAACCTTCA AAGGAGCTGA
      GAAGACCTGG TTGAACCGAT TCCTACCACC TCTTGGAAGT TTCCTCGACT

             oGQ8
      ==========================
      sod-3 prom.  + coding  region
      =======================
      Exon 3
      ===============
                        SacI
                       ~~~~~~~
1751  TGGACACTAT TAAGCCGAGC TCAGAAAAAA TGACTGCTCC AAAGAAGAAG
      ACCTGTGATA ATTCGGCTCG AGTCTTTTTT ACTGACGAGG TTTCTTCTTC

                                                       luc+
                                  ==========================
1801  CGTAAGGTAC CGGTAGAAAA AATGGAAGAC GCCAAAAACA TAAAGAAAGG
```

Fig. 22 continued

```
     GCATTCCATG GCCATCTTTT TTACCTTCTG CGGTTTTTGT ATTTCTTTCC

                                                      luc+
     ==================================================
1851 CCCGGCGCCA TTCTATCCGC TGGAAGATGG AACCGCTGGA GAGCAACTGC
     GGGCCGCGGT AAGATAGGCG ACCTTCTACC TTGGCGACCT CTCGTTGACG

                                                      luc+
     ==================================================
1901 ATAAGGCTAT GAAGAGATAC GCCCTGGTTC CTGGAACAAT TGCTTTTACA
     TATTCCGATA CTTCTCTATG CGGGACCAAG GACCTTGTTA ACGAAAATGT

                                                      luc+
     ==================================================
1951 GATGCACATA TCGAGGTGGA CATCACTTAC GCTGAGTACT TCGAAATGTC
     CTACGTGTAT AGCTCCACCT GTAGTGAATG CGACTCATGA AGCTTTACAG

                                                      luc+
     ==================================================
2001 CGTTCGGTTG GCAGAAGCTA TGAAACGATA TGGGCTGAAT ACAAATCACA
     GCAAGCCAAC CGTCTTCGAT ACTTTGCTAT ACCCGACTTA TGTTTAGTGT

                                                      luc+
     ==================================================
2051 GAATCGTCGT ATGCAGTGAA AACTCTCTTC AATTCTTTAT GCCGGTGTTG
     CTTAGCAGCA TACGTCACTT TTGAGAGAAG TTAAGAAATA CGGCCACAAC

                                                      luc+
     ==================================================
2101 GGCGCGTTAT TTATCGGAGT TGCAGTTGCG CCCGCGAACG ACATTTATAA
     CCGCGCAATA AATAGCCTCA ACGTCAACGC GGGCGCTTGC TGTAAATATT

                                                      luc+
     ==================================================
2151 TGAACGTGAA TTGCTCAACA GTATGGGCAT TTCGCAGCCT ACCGTGGTGT
     ACTTGCACTT AACGAGTTGT CATACCCGTA AAGCGTCGGA TGGCACCACA

                                                      luc+
     ==================================================
2201 TCGTTTCCAA AAAGGGGTTG CAAAAAATTT TGAACGTGCA AAAAAAGCTC
     AGCAAAGGTT TTTCCCCAAC GTTTTTTAAA ACTTGCACGT TTTTTTCGAG

                                                      luc+
     ==================================================
2251 CCAATCATCC AAAAAATTAT TATCATGGAT TCTAAAACGG ATTACCAGGG
     GGTTAGTAGG TTTTTTAATA ATAGTACCTA AGATTTTGCC TAATGGTCCC

                                                      luc+
     ==================================================
2301 ATTTCAGTCG ATGTACACGT TCGTCACATC TCATCTACCT CCCGGTTTTA
     TAAAGTCAGC TACATGTGCA AGCAGTGTAG AGTAGATGGA GGGCCAAAAT

                                                      luc+
     ==================================================
```

Fig. 22 Continued

```
2351  ATGAATACGA TTTTGTGCCA GAGTCCTTCG ATAGGGACAA GACAATTGCA
      TACTTATGCT AAAACACGGT CTCAGGAAGC TATCCCTGTT CTGTTAACGT

                                                     luc+
      ==================================================
2401  CTGATCATGA ACTCCTCTGG ATCTACTGGT CTGCCTAAAG GTGTCGCTCT
      GACTAGTACT TGAGGAGACC TAGATGACCA GACGGATTTC CACAGCGAGA

                                                     luc+
      ==================================================
2451  GCCTCATAGA ACTGCCTGCG TGAGATTCTC GCATGCCAGA GATCCTATTT
      CGGAGTATCT TGACGGACGC ACTCTAAGAG CGTACGGTCT CTAGGATAAA

                                                     luc+
      ==================================================
2501  TTGGCAATCA AATCATTCCG GATACTGCGA TTTTAAGTGT TGTTCCATTC
      AACCGTTAGT TTAGTAAGGC CTATGACGCT AAAATTCACA ACAAGGTAAG

                                                     luc+
      ==================================================
2551  CATCACGGTT TTGGAATGTT TACTACACTC GGATATTTGA TATGTGGATT
      GTAGTGCCAA AACCTTACAA ATGATGTGAG CCTATAAACT ATACACCTAA

                                                    luc+
      ==================================================
2601  TCGAGTCGTC TTAATGTATA GATTTGAAGA AGAGCTGTTT CTGAGGAGCC
      AGCTCAGCAG AATTACATAT CTAAACTTCT TCTCGACAAA GACTCCTCGG

      luc+
      ==================================================
2651  TTCAGGATTA CAAGATTCAA AGTGCGCTGC TGGTGCCAAC CCTATTCTCC
      AAGTCCTAAT GTTCTAAGTT TCACGCGACG ACCACGGTTG GGATAAGAGG

      luc+
      ==================================================
2701  TTCTTCGCCA AAAGCACTCT GATTGACAAA TACGATTTAT CTAATTTACA
      AAGAAGCGGT TTTCGTGAGA CTAACTGTTT ATGCTAAATA GATTAAATGT

      luc+
      ==================================================
2751  CGAAATTGCT TCTGGTGGCG CTCCCCTCTC TAAGGAAGTC GGGGAAGCGG
      GCTTTAACGA AGACCACCGC GAGGGGAGAG ATTCCTTCAG CCCCTTCGCC

      luc+
      ==================================================
2801  TTGCCAAGAG GTTCCATCTG CCAGGTATCA GGCAAGGATA TGGGCTCACT
      AACGGTTCTC CAAGGTAGAC GGTCCATAGT CCGTTCCTAT ACCCGAGTGA

      luc+
      ==================================================
2851  GAGACTACAT CAGCTATTCT GATTACACCC GAGGGGGATG ATAAACCGGG
      CTCTGATGTA GTCGATAAGA CTAATGTGGG CTCCCCCTAC TATTTGGCCC

      luc+
```

Fig. 22 continued

```
     ==================================================
2901 CGCGGTCGGT AAAGTTGTTC CATTTTTTGA AGCGAAGGTT GTGGATCTGG
     GCGCCAGCCA TTTCAACAAG GTAAAAAACT TCGCTTCCAA CACCTAGACC

     luc+
     ==================================================
2951 ATACCGGGAA AACGCTGGGC GTTAATCAAA GAGGCGAACT GTGTGTGAGA
     TATGGCCCTT TTGCGACCCG CAATTAGTTT CTCCGCTTGA CACACACTCT

     luc+
     ==================================================
3001 GGTCCTATGA TTATGTCCGG TTATGTAAAC AATCCGGAAG CGACCAACGC
     CCAGGATACT AATACAGGCC AATACATTTG TTAGGCCTTC GCTGGTTGCG

     luc+
     ==================================================
3051 CTTGATTGAC AAGGATGGAT GGCTACATTC TGGAGACATA GCTTACTGGG
     GAACTAACTG TTCCTACCTA CCGATGTAAG ACCTCTGTAT CGAATGACCC

     luc+
     ==================================================
3101 ACGAAGACGA ACACTTCTTC ATCGTTGACC GCCTGAAGTC TCTGATTAAG
     TGCTTCTGCT TGTGAAGAAG TAGCAACTGG CGGACTTCAG AGACTAATTC

     luc+
     ==================================================
3151 TACAAAGGCT ATCAGGTGGC TCCCGCTGAA TTGGAATCCA TCTTGCTCCA
     ATGTTTCCGA TAGTCCACCG AGGGCGACTT AACCTTAGGT AGAACGAGGT

     luc+
     ==================================================
3201 ACACCCCAAC ATCTTCGACG CAGGTGTCGC AGGTCTTCCC GACGATGACG
     TGTGGGGTTG TAGAAGCTGC GTCCACAGCG TCCAGAAGGG CTGCTACTGC

     luc+
     ==================================================
3251 CCGGTGAACT TCCCGCCGCC GTTGTTGTTT TGGAGCACGG AAAGACGATG
     GGCCACTTGA AGGGCGGCGG CAACAACAAA ACCTCGTGCC TTTCTGCTAC

     luc+
     ==================================================
3301 ACGGAAAAAG AGATCGTGGA TTACGTCGCC AGTCAAGTAA CAACCGCGAA
     TGCCTTTTTC TCTAGCACCT AATGCAGCGG TCAGTTCATT GTTGGCGCTT

     luc+
     ==================================================
3351 AAAGTTGCGC GGAGGAGTTG TGTTTGTGGA CGAAGTACCG AAAGGTCTTA
     TTTCAACGCG CCTCCTCAAC ACAAACACCT GCTTCATGGC TTTCCAGAAT

     luc+
     ==================================================
3401 CCGGAAAACT CGACGCAAGA AAAATCAGAG AGATCCTCAT AAAGGCCAAG
     GGCCTTTTGA GCTGCGTTCT TTTTAGTCTC TCTAGGAGTA TTTCCGGTTC
```

Fig. 22 continued

```
       luc+                                          unc-54  3' UTR
       =========================                    ==============
3451   AAGGGCGGAA AGATCGCCGT GTAATTCTAG GAATTCCAAC TGAGCGCCGG
       TTCCCGCCTT TCTAGCGGCA CATTAAGATC CTTAAGGTTG ACTCGCGGCC

                                                     unc-54  3' UTR
       ==================================================
3501   TCGCTACCAT TACCAACTTG TCTGGTGTCA AAAATAATAG GGGCCGCTGT
       AGCGATGGTA ATGGTTGAAC AGACCACAGT TTTTATTATC CCCGGCGACA

                                                     unc-54  3' UTR
       ==================================================
3551   CATCAGAGTA AGTTTAAACT GAGTTCTACT AACTAACGAG TAATATTTAA
       GTAGTCTCAT TCAAATTTGA CTCAAGATGA TTGATTGCTC ATTATAAATT

                                                     unc-54  3' UTR
       ==================================================
3601   ATTTTCAGCA TCTCGCGCCC GTGCCTCTGA CTTCTAAGTC CAATTACTCT
       TAAAAGTCGT AGAGCGCGGG CACGGAGACT GAAGATTCAG GTTAATGAGA

                                                     unc-54  3' UTR
       ==================================================
3651   TCAACATCCC TACATGCTCT TTCTCCCTGT GCTCCCACCC CCTATTTTTG
       AGTTGTAGGG ATGTACGAGA AAGAGGGACA CGAGGGTGGG GGATAAAAAC

                                                     unc-54  3' UTR
       ==================================================
3701   TTATTATCAA AAAAACTTCT TCTTAATTTC TTTGTTTTTT AGCTTCTTTT
       AATAATAGTT TTTTTGAAGA AGAATTAAAG AAACAAAAAA TCGAAGAAAA

                                                     unc-54  3' UTR
       ==================================================
3751   AAGTCACCTC TAACAATGAA ATTGTGTAGA TTCAAAAATA GAATTAATTC
       TTCAGTGGAG ATTGTTACTT TAACACATCT AAGTTTTTAT CTTAATTAAG

                                                     unc-54  3' UTR
       ==================================================
3801   GTAATAAAAA GTCGAAAAAA ATTGTGCTCC CTCCCCCCAT TAATAATAAT
       CATTATTTTT CAGCTTTTTT TAACACGAGG GAGGGGGGTA ATTATTATTA

                                                     unc-54  3' UTR
       ==================================================
3851   TCTATCCCAA AATCTACACA ATGTTCTGTG TACACTTCTT ATGTTTTTTT
       AGATAGGGTT TTAGATGTGT TACAAGACAC ATGTGAAGAA TACAAAAAAA

              unc-54  3' UTR
       ==================================================
3901   TACTTCTGAT AAATTTTTTT TGAAACATCA TAGAAAAAAC CGCACACAAA
       ATGAAGACTA TTTAAAAAAA ACTTTGTAGT ATCTTTTTTG GCGTGTGTTT

       unc-54 3'  UTR
       ==================================================
3951   ATACCTTATC ATATGTTACG TTTCAGTTTA TGACCGCAAT TTTTATTTCT
       TATGGAATAG TATACAATGC AAAGTCAAAT ACTGGCGTTA AAAATAAAGA
```

Fig. 22 continued

```
         unc-54 3'  UTR
         ==================================================
4001     TCGCACGTCT GGGCCTCTCA TGACGTCAAA TCATGCTCAT CGTGAAAAAG
         AGCGTGCAGA CCCGGAGAGT ACTGCAGTTT AGTACGAGTA GCACTTTTTC

         unc-54 3'  UTR
         ==================================================
4051     TTTTGGAGTA TTTTTGGAAT TTTTCAATCA AGTGAAAGTT TATGAAATTA
         AAAACCTCAT AAAAACCTTA AAAAGTTAGT TCACTTTCAA ATACTTTAAT

         unc-54 3'  UTR
         ==================================================
4101     ATTTTCCTGC TTTTGCTTTT TGGGGGTTTC CCCTATTGTT TGTCAAGAGT
         TAAAAGGACG AAAACGAAAA ACCCCCAAAG GGGATAACAA ACAGTTCTCA

         unc-54 3'  UTR
         ==================================================
4151     TTCGAGGACG GCGTTTTTCT TGCTAAAATC ACAAGTATTG ATGAGCACGA
         AAGCTCCTGC CGCAAAAAGA ACGATTTTAG TGTTCATAAC TACTCGTGCT

         unc-54 3'  UTR
         ==================================================
4201     TGCAAGAAAG ATCGGAAGAA GGTTTGGGTT TGAGGCTCAG TGGAAGGTGA
         ACGTTCTTTC TAGCCTTCTT CCAAACCCAA ACTCCGAGTC ACCTTCCACT

         unc-54 3'  UTR
         ==================================================
4251     GTAGAAGTTG ATAATTTGAA AGTGGAGTAG TGTCTATGGG GTTTTTGCCT
         CATCTTCAAC TATTAAACTT TCACCTCATC ACAGATACCC CAAAAACGGA

         unc-54 3'  UTR                                 MSC II
         ============================================    ===
4301     TAAATGACAG AATACATTCC CAATATACCA AACATAACTG TTTCCTACTA
         ATTTACTGTC TTATGTAAGG GTTATATGGT TTGTATTGAC AAAGGATGAT

            MSC II
         ================
4351     GTCGGCCGTA CGGGCCCTTT CGTCTCGCGC GTTTCGGTGA TGACGGTGAA
         CAGCCGGCAT GCCCGGGAAA GCAGAGCGCG CAAAGCCACT ACTGCCACTT

4401     AACCTCTGAC ACATGCAGCT CCCGGAGACG GTCACAGCTT GTCTGTAAGC
         TTGGAGACTG TGTACGTCGA GGGCCTCTGC CAGTGTCGAA CAGACATTCG

4451     GGATGCCGGG AGCAGACAAG CCCGTCAGGG CGCGTCAGCG GGTGTTGGCG
         CCTACGGCCC TCGTCTGTTC GGGCAGTCCC GCGCAGTCGC CCACAACCGC

4501     GGTGTCGGGG CTGGCTTAAC TATGCGGCAT CAGAGCAGAT TGTACTGAGA
         CCACAGCCCC GACCGAATTG ATACGCCGTA GTCTCGTCTA ACATGACTCT

4551     GTGCACCATA TGCGGTGTGA AATACCGCAC AGATGCGTAA GGAGAAAATA
         CACGTGGTAT ACGCCACACT TTATGGCGTG TCTACGCATT CCTCTTTTAT

4601     CCGCATCAGG CGGCCTTAAG GGCCTCGTGA TACGCCTATT TTTATAGGTT
```

Fig. 22 continued

```
     GGCGTAGTCC GCCGGAATTC CCGGAGCACT ATGCGGATAA AAATATCCAA

4651 AATGTCATGA TAATAATGGT TTCTTAGACG TCAGGTGGCA CTTTTCGGGG
     TTACAGTACT ATTATTACCA AAGAATCTGC AGTCCACCGT GAAAAGCCCC

4701 AAATGTGCGC GGAACCCCTA TTTGTTTATT TTTCTAAATA CATTCAAATA
     TTTACACGCG CCTTGGGGAT AAACAAATAA AAAGATTTAT GTAAGTTTAT

4751 TGTATCCGCT CATGAGACAA TAACCCTGAT AAATGCTTCA ATAATATTGA
     ACATAGGCGA GTACTCTGTT ATTGGGACTA TTTACGAAGT TATTATAACT

                                                      amp
                         ==============================
4801 AAAAGGAAGA GTATGAGTAT TCAACATTTC CGTGTCGCCC TTATTCCCTT
     TTTTCCTTCT CATACTCATA AGTTGTAAAG GCACAGCGGG AATAAGGGAA

                                                      amp
     ==================================================
4851 TTTTGCGGCA TTTTGCCTTC CTGTTTTTGC TCACCCAGAA ACGCTGGTGA
     AAAACGCCGT AAAACGGAAG GACAAAAACG AGTGGGTCTT TGCGACCACT

                                                      amp
     ==================================================
4901 AAGTAAAAGA TGCTGAAGAT CAGTTGGGTG CACGAGTGGG TTACATCGAA
     TTCATTTTCT ACGACTTCTA GTCAACCCAC GTGCTCACCC AATGTAGCTT

                                                      amp
     ==================================================
4951 CTGGATCTCA ACAGCGGTAA GATCCTTGAG AGTTTTCGCC CCGAAGAACG
     GACCTAGAGT TGTCGCCATT CTAGGAACTC TCAAAAGCGG GGCTTCTTGC

                                                      amp
     ==================================================
5001 TTTTCCAATG ATGAGCACTT TTAAAGTTCT GCTATGTGGC GCGGTATTAT
     AAAAGGTTAC TACTCGTGAA AATTTCAAGA CGATACACCG CGCCATAATA

                                                      amp
     ==================================================
5051 CCCGTATTGA CGCCGGGCAA GAGCAACTCG GTCGCCGCAT ACACTATTCT
     GGGCATAACT GCGGCCCGTT CTCGTTGAGC CAGCGGCGTA TGTGATAAGA

                                                      amp
     ==================================================
5101 CAGAATGACT TGGTTGAGTA CTCACCAGTC ACAGAAAAGC ATCTTACGGA
     GTCTTACTGA ACCAACTCAT GAGTGGTCAG TGTCTTTTCG TAGAATGCCT

                                                      amp
     ==================================================
5151 TGGCATGACA GTAAGAGAAT TATGCAGTGC TGCCATAACC ATGAGTGATA
     ACCGTACTGT CATTCTCTTA ATACGTCACG ACGGTATTGG TACTCACTAT

                                              amp
     ==================================================
5201 ACACTGCGGC CAACTTACTT CTGACAACGA TCGGAGGACC GAAGGAGCTA
```

Fig. 22 continued

```
     TGTGACGCCG GTTGAATGAA GACTGTTGCT AGCCTCCTGG CTTCCTCGAT

     amp
     ==================================================
5251 ACCGCTTTTT TGCACAACAT GGGGGATCAT GTAACTCGCC TTGATCGTTG
     TGGCGAAAAA ACGTGTTGTA CCCCCTAGTA CATTGAGCGG AACTAGCAAC

     amp
     ==================================================
5301 GGAACCGGAG CTGAATGAAG CCATACCAAA CGACGAGCGT GACACCACGA
     CCTTGGCCTC GACTTACTTC GGTATGGTTT GCTGCTCGCA CTGTGGTGCT

     amp
     ==================================================
5351 TGCCTGTAGC AATGGCAACA ACGTTGCGCA AACTATTAAC TGGCGAACTA
     ACGGACATCG TTACCGTTGT TGCAACGCGT TTGATAATTG ACCGCTTGAT

     amp
     ==================================================
5401 CTTACTCTAG CTTCCCGGCA ACAATTAATA GACTGGATGG AGGCGGATAA
     GAATGAGATC GAAGGGCCGT TGTTAATTAT CTGACCTACC TCCGCCTATT

     amp
     ==================================================
5451 AGTTGCAGGA CCACTTCTGC GCTCGGCCCT TCCGGCTGGC TGGTTTATTG
     TCAACGTCCT GGTGAAGACG CGAGCCGGGA AGGCCGACCG ACCAAATAAC

     amp
     ==================================================
5501 CTGATAAATC TGGAGCCGGT GAGCGTGGGT CTCGCGGTAT CATTGCAGCA
     GACTATTTAG ACCTCGGCCA CTCGCACCCA GAGCGCCATA GTAACGTCGT

     amp
     ==================================================
5551 CTGGGGCCAG ATGGTAAGCC CTCCCGTATC GTAGTTATCT ACACGACGGG
     GACCCCGGTC TACCATTCGG GAGGGCATAG CATCAATAGA TGTGCTGCCC

     amp
     ==================================================
5601 GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT GAGATAGGTG
     CTCAGTCCGT TGATACCTAC TTGCTTTATC TGTCTAGCGA CTCTATCCAC

     amp
     ====================
5651 CCTCACTGAT TAAGCATTGG TAACTGTCAG ACCAAGTTTA CTCATATATA
     GGAGTGACTA ATTCGTAACC ATTGACAGTC TGGTTCAAAT GAGTATATAT

5701 CTTTAGATTG ATTTAAAACT TCATTTTTAA TTTAAAAGGA TCTAGGTGAA
     GAAATCTAAC TAAATTTTGA AGTAAAAATT AAATTTTCCT AGATCCACTT

5751 GATCCTTTTT GATAATCTCA TGACCAAAAT CCCTTAACGT GAGTTTTCGT
     CTAGGAAAAA CTATTAGAGT ACTGGTTTTA GGGAATTGCA CTCAAAAGCA

5801 TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC TTCTTGAGAT
```

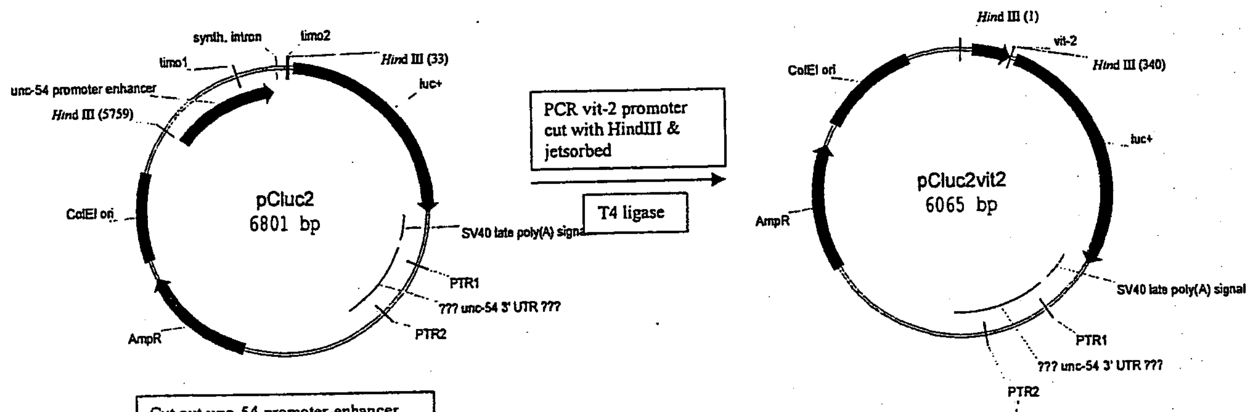
Fig 22. continued

```
      AGGTGACTCG CAGTCTGGGG CATCTTTTCT AGTTTCCTAG AAGAACTCTA

5851  CCTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA AACCACCGCT
      GGAAAAAAAG ACGCGCATTA GACGACGAAC GTTTGTTTTT TTGGTGGCGA

5901  ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCAACT CTTTTTCCGA
      TGGTCGCCAC CAAACAAACG GCCTAGTTCT CGATGGTTGA GAAAAAGGCT

5951  AGGTAACTGG CTTCAGCAGA GCGCAGATAC CAAATACTGT CCTTCTAGTG
      TCCATTGACC GAAGTCGTCT CGCGTCTATG GTTTATGACA GGAAGATCAC

6001  TAGCCGTAGT TAGGCCACCA CTTCAAGAAC TCTGTAGCAC CGCCTACATA
      ATCGGCATCA ATCCGGTGGT GAAGTTCTTG AGACATCGTG GCGGATGTAT

6051  CCTCGCTCTG CTAATCCTGT TACCAGTGGC TGCTGCCAGT GGCGATAAGT
      GGAGCGAGAC GATTAGGACA ATGGTCACCG ACGACGGTCA CCGCTATTCA

6101  CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA TAAGGCGCAG
      GCACAGAATG GCCCAACCTG AGTTCTGCTA TCAATGGCCT ATTCCGCGTC

6151  CGGTCGGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT TGGAGCGAAC
      GCCAGCCCGA CTTGCCCCCC AAGCACGTGT GTCGGGTCGA ACCTCGCTTG

6201  GACCTACACC GAACTGAGAT ACCTACAGCG TGAGCATTGA GAAAGCGCCA
      CTGGATGTGG CTTGACTCTA TGGATGTCGC ACTCGTAACT CTTTCGCGGT

6251  CGCTTCCCGA AGGGAGAAAG GCGGACAGGT ATCCGGTAAG CGGCAGGGTC
      GCGAAGGGCT TCCCTCTTTC CGCCTGTCCA TAGGCCATTC GCCGTCCCAG

6301  GGAACAGGAG AGCGCACGAG GGAGCTTCCA GGGGGAAACG CCTGGTATCT
      CCTTGTCCTC TCGCGTGCTC CCTCGAAGGT CCCCCTTTGC GGACCATAGA

6351  TTATAGTCCT GTCGGGTTTC GCCACCTCTG ACTTGAGCGT CGATTTTTGT
      AATATCAGGA CAGCCCAAAG CGGTGGAGAC TGAACTCGCA GCTAAAAACA

6401  GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG CAACGCGGCC
      CTACGAGCAG TCCCCCCGCC TCGGATACCT TTTTGCGGTC GTTGCGCCGG

6451  TTTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA TGTTCTTTCC
      AAAAATGCCA AGGACCGGAA AACGACCGGA AAACGAGTGT ACAAGAAAGG

6501  TGCGTTATCC CCTGATTCTG TGGATAACCG TATTACCGCC TTTGAGTGAG
      ACGCAATAGG GGACTAAGAC ACCTATTGGC ATAATGGCGG AAACTCACTC

6551  CTGATACCGC TCGCCGCAGC CGAACGACCG AGCGCAGCGA GTCAGTGAGC
      GACTATGGCG AGCGGCGTCG GCTTGCTGGC TCGCGTCGCT CAGTCACTCG

6601  GAGGAAGCGG AAGAGCGCCC AATACGCAAA CCGCCTCTCC CCGCGCGTTG
      CTCCTTCGCC TTCTCGCGGG TTATGCGTTT GGCGGAGAGG GGCGCGCAAC

6651  GCCGATTCAT TAATGCAGCT GGCACGACAG GTTTCCCGAC TGGAAAGCGG
      CGGCTAAGTA ATTACGTCGA CCGTGCTGTC CAAAGGGCTG ACCTTTCGCC

6701  GCAGTGAGCG CAACGCAATT AATGTGAGTT AGCTCACTCA TTAGGCACCC
```

Fig-22 continued

```
     CGTCACTCGC GTTGCGTTAA TTACACTCAA TCGAGTGAGT AATCCGTGGG

6751 CAGGCTTTAC ACTTTATGCT TCCGGCTCGT ATGTTGTGTG GAATTGTGAG
     GTCCGAAATG TGAAATACGA AGGCCGAGCA TACAACACAC CTTAACACTC

6801 CGGATAACAA TTTCACACAG GAAACAGCTA TGACCATGAT TACGCCAAGC
     GCCTATTGTT AAAGTGTGTC CTTTGTCGAT ACTGGTACTA ATGCGGTTCG

6851 TGTAAGTTTA AACATGATCT TACTAACTAA CTATTCTCAT TTAAATTTTC
     ACATTCAAAT TTGTACTAGA ATGATTGATT GATAAGAGTA AATTTAAAAG

6901 AGAGCTTAAA AATGGCTGAA ATCACTCACA ACGATGGATA CGCTAACAAC
     TCTCGAATTT TTACCGACTT TAGTGAGTGT TGCTACCTAT GCGATTGTTG

                                    PstI
                                   ~~~~~
6951 TTGGAAATGA AATAAGCTTG CATGCCTGCA
     AACCTTTACT TTATTCGAAC GTACGGACGT
```

Figure 23



Cut out unc-54 promoter-enhancer from pCluc2

Determine direction of insert by RE cleavage XbaI/NheI NO INTRON

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(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG)*
— *of inventorship (Rule 4.17(iv)) for US only*

Published:
— *with international search report*
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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/93669 A3

(54) Title: COMPOUND SCREENS RELATING TO INSULIN DEFICIENCY OR INSULIN RESISTANCE

(57) Abstract: The invention is concerned with use of the model organism *C. elegans* as a research tool to screen for compounds active in insulin signalling. In particular, the invention relates to improved screening methods based on release of *C. elegans* from the dauer larval state.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 01/01199

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 51351 A (GEN HOSPITAL CORP) 19 November 1998 (1998-11-19) cited in the application claims 1-8	1-62
A	GEMS DAVID ET AL: "Two pleiotropic classes of daf-2 mutation affect larval arrest, adult behavior, reproduction and longevity in Caenorhabditis elegans." GENETICS, vol. 150, no. 1, 1998, pages 129-155, XP002191748 ISSN: 0016-6731 cited in the application the whole document	

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
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- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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- "&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 February 2002	15/03/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Niemann, F

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	GIL E B ET AL: "REGULATION OF THE INSULIN-LIKE DEVELOPMENTAL PATHWAY OF CAENORHABDITIS ELEGANS BY A HOMOLOG OF THE PTEN TUMOR SUPPRESSOR GENE" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 96, March 1999 (1999-03), pages 2925-2930, XP002926980 ISSN: 0027-8424 abstract	
A	KIMURA K D ET AL: "DAF-2, AN INSULIN RECEPTOR-LIKE GENE THAT REGULATES LONGEVITY AND DIAPAUSE IN CAENORHABDITIS ELEGANS" SCIENCE, AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, US, vol. 277, 15 August 1997 (1997-08-15), pages 942-946, XP002910188 ISSN: 0036-8075 cited in the application the whole document	
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IB 01/01199

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
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			AU	7494198	A	08-12-1998
			EP	1019092	A1	19-07-2000
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			EP	1163515	A1	19-12-2001
			WO	0033068	A1	08-06-2000

Applicant(s): HOPPE, et al.
Serial No.: 10/766,339
Filing Date: 1/28/2004
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PRIOR ART